SX20 Hardware User Guide

This section provides a complete guide to the hardware of the SX20 stopped flow system. It includes a technical description of the hardware, instrument set-up, hardware-related aspects of operation and a basic maintenance guide.

Applied Photophysics Limited
203/205 Kingston Road
Leatherhead KT22 7PB
United Kingdom

Tel: +44 (0)1372 386537
(USA) 1- 800 543 4130
Fax: +44 (0)1372 386477

Email: techsup@photophysics.com
URL: www.photophysics.com

Contents

2.0 Introduction 5
2.1.1 Description 5
2.1.2 Components 5
2.2 SX20 Optional Accessories 7
2.2.1 SQ.1 Sequential (Double) Mixing Option 7
2.2.2 SEM.1 Emission Scanning Option 7
2.2.3 AM.1 Extended Absorbance Option 7
2.2.4 PDA.1 Photodiode Array Option 8
2.2.5 UV.1 Deuterium Light Source Option 8
2.2.6 DD.1 Dual Channel Detection Option 8
2.2.7 DF.1 Dual Channel Fluorescence Detection Option 9
2.2.8 FP.1 Fluorescence Polarisation Option 9
2.2.9 RC.1 5μL Rapid Kinetics Cell Option 9
2.2.10 QFA.1 Quench Flow Adaptor Option 9
2.2.11 AN.1 Anaerobic Accessory 9
2.3 SX20 Instrument Set-up 10
2.3.1 Site Requirements 10
2.3.2 SX20 Hardware Set-up 11
2.3.3 SX20 Electrical Set-up 15
2.3.4 SX20 Optional Accessories Basic Set-up 20
2.3.4.1 SEM.1 Scanning Emission Monochromator Option 20
2.3.4.2 AM.1 Extended Absorbance Option 21
2.4 SX20 Hardware Operation 22
2.4.1 Sample Handling Unit 22
2.4.1.1 Introduction 22
2.4.1.2 Description of a Stopped-Flow Drive 24
2.4.1.3 Stopped-Flow Circuit 24
2.4.1.4 Drive Syringes 26
2.4.1.5 Drive Valves 26
2.4.1.6 Stop Syringe 27
2.4.1.7 Stop Valve 28
2.4.1.8 Flow Tubing 29
2.4.1.9 Interchangeable Cell Cartridge System 32
5.1.10 Optical Cells 34
2.4.1.11 Absorbance Pathlength Selection 35
2.4.1.12 The Auto-Stop Mechanism 36
2.4.1.13 Pneumatic Drive and Drive Rams 39
2.4.1.14 Temperature Control 40
2.4.2 Absorbance Detector 42

2.4.2.1 Description....42
2.4.2.2 Basic Set-Up and Electrical Connections....42
2.4.2.3 PMT Characteristics....43
2.4.3 Fluorescence Detector....44
2.4.3.1 Description....44
2.4.3.2 Basic Set-Up and Electrical Connections....44
2.4.3.3 PMT Characteristics....45
2.4.3.4 Dual Channel Fluorescence Set-Up....46
2.4.4 Monochromator....47
2.4.4.1 Description....47
2.4.4.2 Basic Set-Up and Electrical Connections....48
2.4.4.3 Selecting a Suitable Monochromator Slit Width (Band Pass)....48
2.4.4.4 Band Pass Calculation....48
2.4.4.5 Dual Monochromator Operation....49
2.4.4.6 Photodiode Array Operation....49
2.4.4.7 Absorbance Measurements in the near IR (>650nm)....49
2.4.4.8 Maintenance....49
2.4.5 150W Lamp Housing....50
2.4.5.1 Description....50
2.4.5.2 Basic Set-Up and Electrical Connections....51
2.4.5.3 Lamp Stability Magnet....51
2.4.5.4 Lamp Types....52
2.4.5.5 Purging the Lamp Housing (Ozone-Producing Lamp Only)....54
2.4.5.6 Lamp Alignment Procedure....56
2.4.5.7 Lamp Lifetime....57
2.4.5.8 Lamp Stability Test....57
2.4.6 Lamp Power Supply Unit....58
2.4.6.1 Description....58
2.4.6.2 Basic Set-Up and Electrical Connections....58
2.4.6.3 Operation....59
2.4.6.4 Use of Xenon and Mercury-Xenon Lamps....59
2.4.6.5 Maintenance....60
2.4.6.6 Electrical Characteristics....60
2.4.7 Electronics Unit....61
2.4.7.1 Description....61
2.4.7.2 Operation....64
2.4.7.3 Maintenance....65
2.4.7.4 Electrical Characteristics....65
2.4.8 Workstation....66
2.4.8.1 Description....66
2.4.8.2 Basic Set-Up and Electrical Connections....66
2.4.8.3 Operation....67
2.4.9 Photo Diode Array Detector (Option PDA.1)....68

2.4.9.1 Description....68
2.4.9.2 Basic Set-Up and Electrical Connections....68
2.5 SX20 Experimental Operation.... 69
2.5.1 Single Mixing Stopped-Flow Operation....69
2.5.2 Sequential Mixing Stopped-Flow Operation....73
2.5.3 Optical Filters....78
2.5.3.1 Absorbance Measurements in the near IR (>650nm)....78
2.5.3.2 Fluorescence Measurements....78
2.5.4 Asymmetric (Variable Ratio) Mixing....81
2.5.4.1 Setting the Drive Pressure for Asymmetric Mixing Experiments....81
2.5.4.2 Setting the Drive Volume for Asymmetric Mixing Experiments....82
2.5.5 Anaerobic Operation (using option AN.1)....83
2.5.5.1 Purging the Drive Syringes....83
2.5.5.2 Flow Circuit Preparation....84
2.5.5.3 Sample Introduction Care....84
2.5.5.4 Procedure for Bench Top Anaerobic Stopped-Flow Studies....85

2.0 Introduction

This is a reference guide to the hardware of the SX20 stopped-flow reaction analyser. It describes the set-up, operation and routine maintenance of the hardware of the instrument. A trouble-shooting guide is also included to help the user fault find commonly encountered problems.

2.1 SX20 Stopped-Flow Reaction Analyser

This section describes the experimental capabilities of the standard configuration SX20 stopped-flow reaction analyser and the components that comprise the system.

2.1.1 Description

The SX20 stopped-flow system in its standard configuration, without any of the optional accessories described later in this section allows the user to carry out single mixing stopped-flow measurements. The user can select between detection of absorbance kinetics at a single wavelength or fluorescence kinetics using an optical filter to isolate the fluorescence signal.

2.1.2 Components

The standard configuration SX20 stopped-flow system consists of the following major hardware components: -

1) 150W Xenon lamp power supply and igniter unit
2) 150W Xenon lamp housing (lamp fitted)
3) Monochromator (Mono 1)
4) Optical rail for mounting of the lamp housing and monochromator
5) Fibre optic light guide
6) Sample handling unit fitted with 20µL cell cartridge
7) Absorbance detector
8) Fluorescence detector
9) Electronics Unit fitted with single detection channel
10) Computer fitted with fibre optic interface card
11) Monitor
12) Printer

These components will be described in further detail later in this section. In addition to the major components, the SX20 system is supplied with the following ancillary components:-

1) Mains electricity distribution board and mains cables
2) Set of cables for electrical connection of the SX20 major components
3) Pneumatic tubing and connectors for connecting the system to an 8bar gas supply
4) In-line pressure regulator for controlling drive pressure
5) Storage case including toolkit and consumable spares kit
6) User manual and back-up software

2.2 SX20 Optional Accessories

This section describes the optional accessories that are available for the SX20 system. These options are available for new systems or as upgrades to existing systems as the research interests of the user evolve.

2.2.1 SQ.1 Sequential (Double) Mixing Option

The sequential (double) mixing option equips the sample handling unit with four drive syringes and permits the mixing of two reagents (A+B) with a first mixing drive and after a user defined delay period, mixes the aged solution with a third reagent (C). This option consists of the following components:-

1) A sequential mixing sample handling unit
2) 2 sequential mixing drive rams
3) Stop syringe brake mechanism

2.2.2 SEM.1 Emission Scanning Option

This emission scanning option extends the fluorescence detection capability of the SX20. An emission monochromator located between the optical cell and the fluorescence detector allows collection of fluorescence kinetic traces at a single wavelength and collection of steady state and time resolved emission spectra. It consists of the following components: -

1) Emission monochromator (Mono 2)
2) Second optical rail and four supports
3) Emission fibre optic light guide

2.2.3 AM.1 Extended Absorbance Option

The extended absorbance option provides improved stray light performance in the far UV enabling accurate absorbance measurements up to 2AU below 250nm. It consists of the following components: -

1) Second monochromator (Mono 2)
2) Extended optical rail
3) Mono 1 to Mono 2 optical coupler

2.2.4 PDA.1 Photodiode Array Option

The Photodiode Array (PDA) option allows collection of time resolved absorbance spectra from a single stopped-flow drive. There are two different versions available: A UV region PDA operates in the wavelength region 180-750nm. A visible region PDA operates in the wavelength range 350-1100nm. The option consists of the following components: -

1) Photodiode array unit (UV or visible version)
2) PDA fibre optic light guide (xenon light source version)
3) Electronics Unit PDA module

2.2.5 UV.1 Deuterium Light Source Option

The deuterium light source option allows the collection of PDA data using the UV version PDA accessory down to 200nm. The option consists of the following components:-

1) Deuterium lamp power supply unit
2) Deuterium lamp housing (lamp fitted)
3) PDA fibre optic light guide (deuterium light source version)

2.2.6 DD.1 Dual Channel Detection Option

The dual channel detection accessory allows fluorescence and absorbance data to be collected simultaneously using the absorbance and fluorescence PMT detectors. It consists of the following components:-

1) Dual channel PMT module fitted in the Electronics Unit.
2) Detector cable for connecting the detector to the PMT module.

2.2.7 DF.1 Dual Channel Fluorescence Detection Option

The dual channel fluorescence detection accessory allows fluorescence data to be collected simultaneously using two fluorescence PMT detectors. It requires the DD.1 dual channel option and consists of the following additional components:-

1) Additional fluorescence detector
2) Fluorescence detector mounting fittings and filter holder

2.2.8 FP.1 Fluorescence Polarisation Option

The fluorescence polarisation accessory allows collection of polarisation and anisotropy signals. It consists of the following components:-

1) Additional fluorescence detector
2) T-format excitation and emission polarizer accessory

2.2.9 RC.1 5μL Rapid Kinetics Cell Option

The 5μL rapid kinetics cell allows collection of stopped-flow data with a dead time of 0.45ms. The cell is mounted in an interchangeable cell cartridge supplied in a protective storage case and is supplied with a 1mL stop syringe.

2.2.10 QFA.1 Quench Flow Adaptor Option

The quench flow accessory utilises the sequential mixing capability of the SHU. The adaptor is mounted in the standard interchangeable cell cartridge and is fitted with a basic sample collection loop.

2.2.11 AN.1 Anaerobic Accessory

The anaerobic accessory improves the ease with which anaerobic experiments can be performed without the need for a glove-box environment. It consists of the following components: -

1) Pack of ten 3-way valves for loading anaerobic samples into the SHU
2) Nitrogen purging manifold that connects to the sample handling unit

2.3 SX20 Instrument Set-up

This section describes the basic set-up procedure and configuration of the SX20 stopped-flow reaction analyser. More detailed information relating to the set-up and connections of each component of the system is provided later in this chapter.

2.3.1 Site Requirements

The SX20 should be set-up in a clean laboratory environment on a sturdy work surface. The laboratory temperature should be maintained within the range 15-35°C.

The minimum space requirement for the SX20 (length x width x height) is as follows:

SX20 system: 2.0m x 0.7m x 0.5m
SX20 system fitted with option AM.1: 2.5m x 0.7m x 0.5m

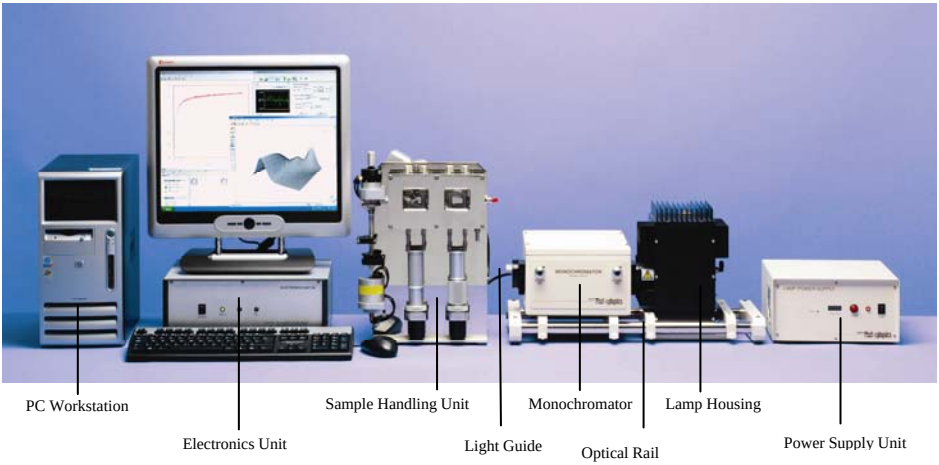
The SX20 requires a stable mains electricity supply of 85-264V.

The sample handling unit is pneumatically driven and requires a compressed gas supply (preferably nitrogen) capable of operating at 8bar (120psi). The supply should be fitted with a push on tube adaptor to accept the flexible hose (6mm internal diameter) that connects to the SHU inlets.

For connection to a water circulator unit, the SHU is provided with an inlet and outlet fitting compatible with a flexible hose of 8mm internal diameter.

2.3.2 SX20 Hardware Set-up

The picture below shows the standard arrangement of the major hardware components of the system.



The major components of the SX20 spectrophotometer should be assembled according to the picture above, with the lamp housing and monochromator positioned on the optical rail to the right of the sample handling unit. (Note: The sample-handling unit is shown with the sequential mixing SQ.1 option fitted).

SX20 systems equipped with the SEM.1 and AM.1 options are supplied with two monochromators and an extended optical rail. The set-up of these systems should be assembled according to the instructions provided later in this Chapter.

The following descriptions are provided to assist the user with the set-up of the major components of the SX20:

Lamp Power Supply Unit

The 150W xenon lamp power supply unit is generally located to the right of the optical rail. Suitable clearance to the rear and left hand side should be allowed for ventilation of this unit.

Xenon Arc Lamp Housing

The 150W xenon lamp housing is mounted on the right hand side of the optical rail. A magnet assembly is sometimes attached to the rear of the housing. This provides magnetic stabilisation of the lamp arc.

Monochromator

The monochromator is located on the left hand side of the optical rail to the right of the 150W xenon arc lamp housing. It is essential that the lamp housing and monochromator are correctly coupled together. The two units must be pushed together so that the exit port of the lamp locates firmly with the entrance port of the monochromator. Both units should be locked in position on the optical rail using the four plastic screws on each unit

Fibre Optic Light Guide

The fibre optic light guide transmits light exiting the monochromator to the optical cell of the stopped-flow sample handling unit. The large coupler of the light guide is pushed into the exit slit assembly of the monochromator and secured using 2x M3 finger screws. The other end is connected to one of the two lower ports of the optical cell block. The short pathlength is accessed from the lower right hand port and the long pathlength is accessed from the lower left hand port. A plastic blanking plug should be placed over the unused port.

Sample Handling Unit

Pneumatic Connections - The stopped flow operation of the sample handling unit is driven by a compressed gas supply. A nitrogen cylinder is recommended for this purpose and must be regulated to 8bar (125psi). A length of flexible tube (6mm internal diameter) is supplied for connecting the cylinder to the sample handling unit.

The cylinder pressure regulator should be fitted with a push-on fitting to accommodate the flexible tube which is clamped onto the fitting with the finger-tightened hose clamp supplied with the system.

The main pneumatic inlets (labelled 1 & 2) are located on the rear of the sample handling unit. Port 1 is internally connected to the auto-stop and, on sample handling units equipped for sequential mixing, the right hand drive ram. Port 2 is connected to the left hand drive ram.

An h-shaped pneumatic tubing arrangement allows both of these ports to be connected to the flexible tube of the compressed gas supply (see picture). The arrangement also incorporates an in-line pressure regulator that is used to adjust the drive pressure of the left hand drive ram.

This connector may additionally be used for supplying nitrogen to the lamp housing when using ozone-producing lamps or when using the anaerobic purging manifold. Detailed instructions for operating with ozone-producing lamps are provided in the xenon lamp operation section of the manual.

Stopped-Flow Circuit - The flow circuit of the sample handling unit requires connection of the waste flow tube to the outlet of the stop valve. Place the free end of the waste tube into a suitable receptacle.

Note – To prevent damage to the optical cell during shipping, the flow circuit is filled with an antifreeze solution. The flow circuit should be flushed thoroughly with distilled water prior to use.

Water Circulator Connections - Two tubing connections on the lower right of the sample handling unit water bath permit connection of a circulating thermostatic water bath to the stopped-flow sample handling unit. The front port is the inlet and the rear port is the outlet. It is important that the inlet and outlet are connected correctly otherwise the sample handling unit water bath will not fill correctly. It is recommended that the circulating thermostatic water bath is positioned below the sample-handling unit to aid the drainage procedure for the water bath.

Absorbance Detector

The absorbance photomultiplier tube detector is connected to one of the two upper ports of the cell housing and opposite the port used for the light input. A plastic blanking plug should be placed over each of the unused ports.

Fluorescence Detector

The fluorescence photomultiplier tube detector is connected to the rear port on the cell housing. The light guide is normally connected to the short pathlength port (lower right hand side) of the cell housing.

Electronics Unit

The electronics unit should be positioned to the left of the sample handling unit.

Workstation, Monitor and Printer

The workstation is normally positioned to the left of the electronics unit. The monitor is positioned above the electronics unit.

2.3.3 SX20 Electrical Set-up

This section describes the basic electrical set-up of the SX20 stopped-flow reaction analyser. It systematically lists the connections that must be made between the various components of the system.

Mains Electricity Supply

The lamp power supply unit and electronics unit feature a universal mains power supply and do not need to be set for the mains electricity voltage of your particular country. These units are provided with mains cables terminated in IEC plugs. These plugs should be connected to the distribution board provided. The distribution board should be fitted with a plug suitable for the mains sockets fitted in your laboratory. This means that only one socket is required to power the entire system.

The workstation PC, monitor and printer are each supplied with a mains cable for connection to the distribution board.

The diagram overleaf shows the mains supply connections.

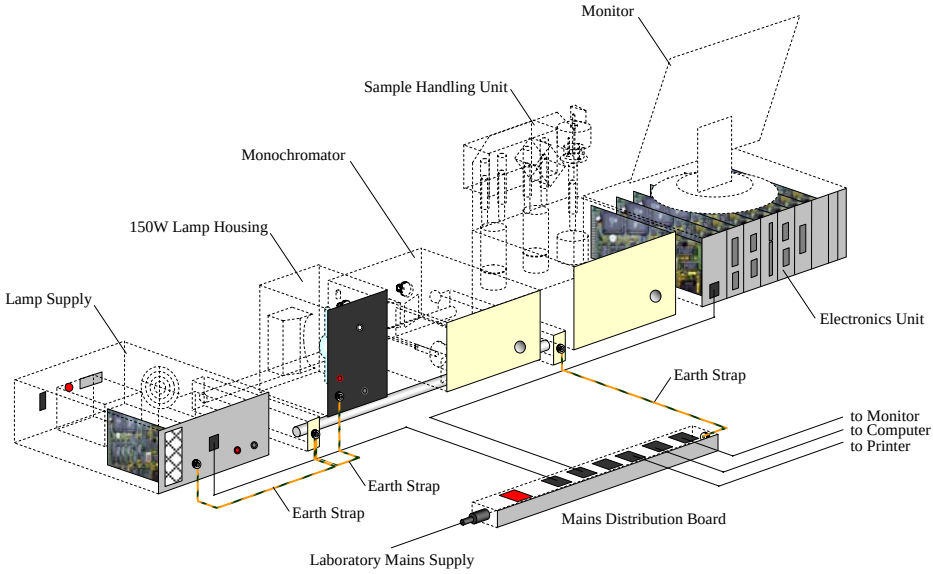
Earth (Ground) Connections

The electronic circuitry used in the stopped-flow spectrometer is very sensitive and must be correctly grounded. The lamp power supply unit, the lamp housing and the mains distribution board all have earth posts that must be connected to the earth post on the optical rail via the braided earth straps provided with the system.

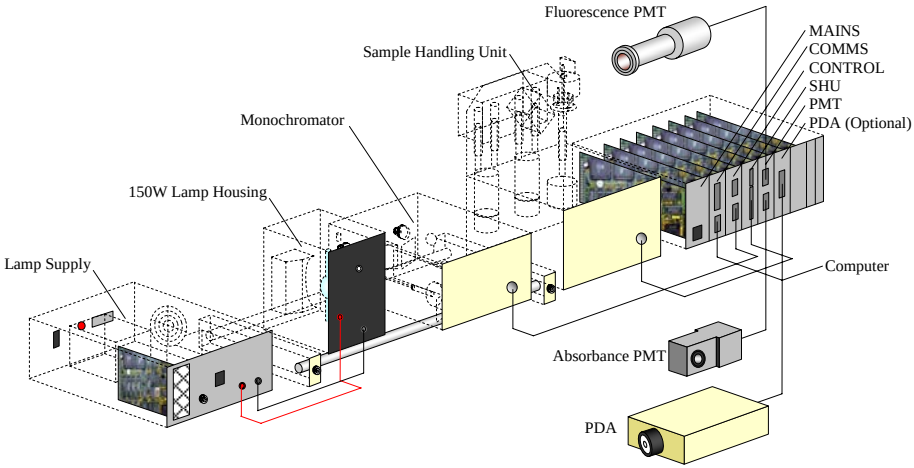
The system is earthed through the mains supply earth. It is vital that the laboratory mains electrical supply where the system will be used is correctly earthed.

The diagram overleaf shows the basic configuration of the earth connections.

Note that the other components of the system are separately earthed.



SX20 mains supply and earth connections



SX20 electrical connections

Lamp Power Supply Unit

One loose, braided cable provides the earth link between the lamp power supply and the optical rail. In addition to the mains inlet socket, there are sockets for two output cables from the safe-start igniter of the lamp power supply. These outputs are connected to the inlet sockets on the lamp housing unit using the loose black and red cables. The black cable is the negative and also carries the 13000V ignition pulse to start the lamp, hence the heavy insulation. The red cable is the positive.

150W Xenon Arc Lamp Housing

One loose, braided cable provides the earth link between the lamp housing and the optical rail. The lamp housing is fitted with two input terminals (black negative and red positive) that are connected to the lamp power supply unit.

Monochromators

For systems with one monochromator, the grey monochromator cable fitted with a 9-pin D-connector is connected to the Drive 1 port on the control module of the electronics unit. For SX20 systems fitted with the SEM.1 or AM.1 option (i.e. two monochromators) Mono 1 must be connected to the Drive 1 port and Mono 2 must be connected to the Drive 2 port on the control module of the Electronics Unit.

Sample Handling Unit

The sample handling unit is connected to the KSHU module in the Electronics Unit via a black cable fitted with a 44-pin D-connector.

Absorbance Detector

The absorbance detector is connected to channel 1 of the PMT module on the electronics rack via a black cable fitted with a 10-pin D-connector.

Fluorescence Detector

The end window fluorescence detector is connected to channel 2 of the PMT module on the electronics rack via a black cable fitted with a 10-pin D-connector.

Electronics Unit

The electronics unit contains a number of plug-in modules for controlling the various operations of the stopped-flow system. It is fitted with one mains supply input. The modules are fitted with a range of sockets and must be connected as follows:

12V and 5V Supply Modules - These modules are not connected to other components on the SX20.

Communications Module - This is connected to the PCI card housed in the workstation PC via a fibre optic communication cable. The Temp Inputs port is currently redundant on the SX20.

Control Module 1 - This provides control of the monochromators. For systems with one monochromator, the monochromator must be connected to the Drive 1 port. For SX20 systems fitted with the SEM.1 or AM.1 options, (i.e. two monochromators), Mono 1 must be connected to the Drive 1 port and Mono 2 must be connected to the Drive 2 port.

KSHU Module - This provides control of the stopped-flow sample handling unit. The sample handling unit is connected via a black cable fitted with a 44-pin D-connector. The Lamp Cont port is currently redundant on the SX20 system.

PMT Module - This provides control of the photomultiplier tubes. There are two versions of the PMT module. The single channel PMT module provides a single channel for fluorescence or absorbance measurement. The detector is connected via a black cable fitted with a 10-pin D-connector. In the case of the dual channel PMT

module, the absorbance detector is connected to Channel 1 via a black cable fitted with a 10-pin D-connector. The fluorescence detector is connected to Channel 2 via a black cable fitted with a 10-pin D-connector.

PDA Module - For systems equipped with the photodiode array option, this module controls the PDA unit which is mounted on the back of the sample handling unit. The PDA unit is connected to the module via a black cable fitted with a 26-pin D-connector.

Workstation, Monitor and Printer

Refer to the user manuals supplied with these units for detailed instructions relating to electrical connections.

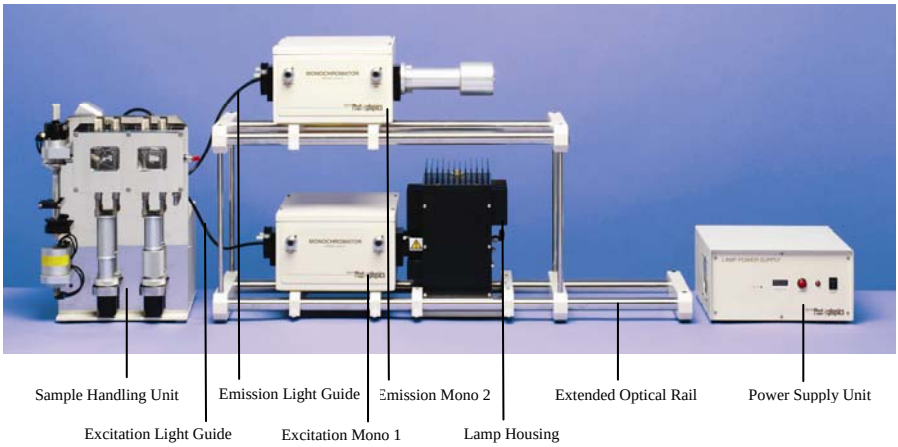
The workstation houses a PCI card that is connected to the communications module of the Electronics Unit via a fibre optic communication cable.

2.3.4 SX20 Optional Accessories Basic Set-up

This section describes the set-up of the optional accessories for the SX20 system. The basic instrument configuration does not alter significantly for any of the optional accessories with the exception of options SEM.1 and AM.1 which feature a second monochromator. Instrument set-up for other optional accessories will be described fully in the relevant operating instructions section.

2.3.4.1 SEM.1 Scanning Emission Monochromator Option

This option features a two-tiered optical rail and second monochromator. The instrument layout for this option is shown in the picture below:



The general assembly of the components is similar to the basic system. However, the following points should be noted:-

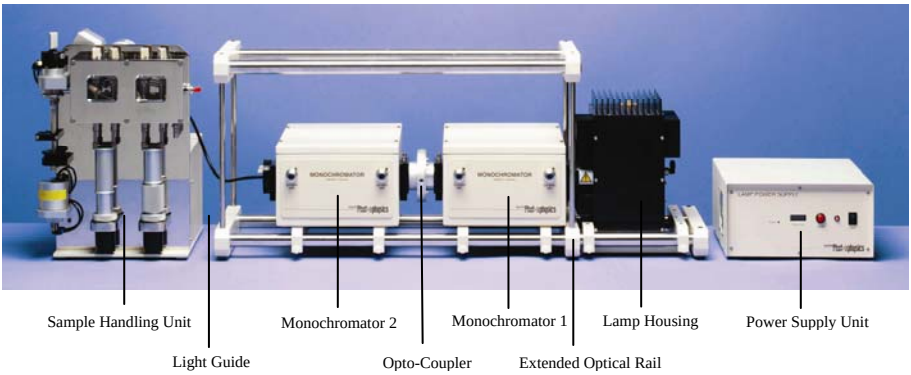
The lamp is always connected to monochromator 1 and never to monochromator 2.

Monochromator 2 is connected to the Drive 2 port of Control Module 1 in the Electronics Unit.

The excitation light guide has a rectangular bundle of fibres of dimensions 5mm x 1mm at the SHU end. The emission light guide has a circular bundle of fibres of 3.5mm diameter at the SHU end.

2.3.4.2 AM.1 Extended Absorbance Option

This option features an extended optical rail and second monochromator. The instrument layout for this option is shown in the picture below:



The general assembly of the components is similar to the basic system. However, the following points should be noted:-

The lamp is always connected to monochromator 1 and never to monochromator 2.

Monochromator 2 is connected to the Drive 2 port of Control Module 1 in the Electronics Unit.

The monochromators are connected using the mono-to-mono opto-coupler.

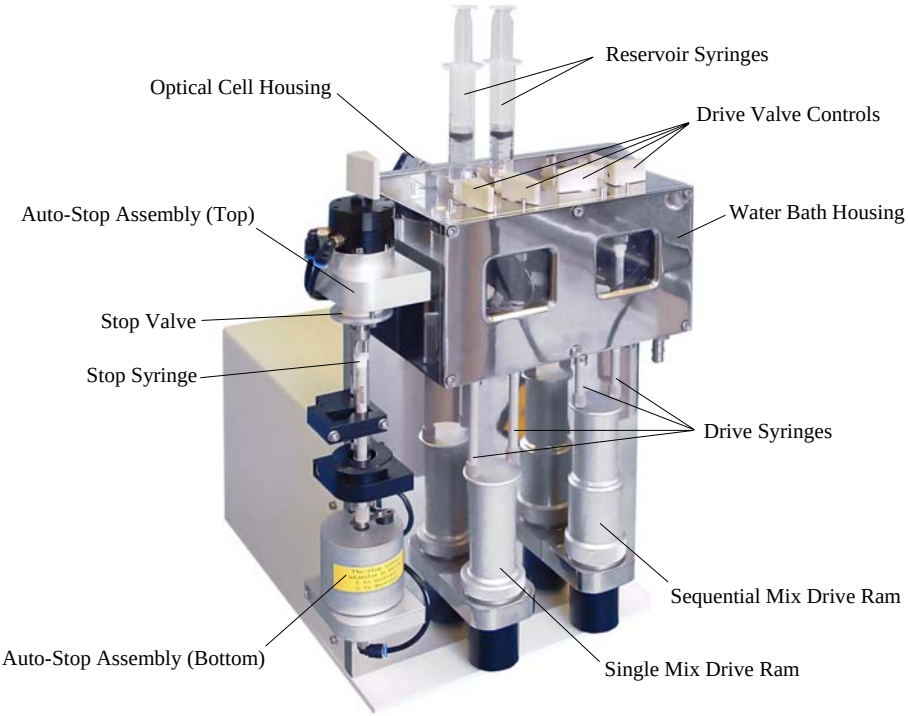
2.4 SX20 Hardware Operation

This section provides detailed information relating to the hardware of the SX20 stopped-flow reaction analyser.

2.4.1 Sample Handling Unit

2.4.1.1 Introduction

The Sample Handling Unit (SHU) is the heart of the SX20 stopped-flow reaction analyser where reagents are loaded, mixed and monitored for a signal change. The picture below shows an SHU equipped with the sequential (double) mixing (SQ.1) option. Principal components of the system are labelled for reference purposes and are described in detail in this section.



The picture overleaf shows the SHU fitted with the sequential (double) mixing option. This option equips the SHU with 4 drive syringes and 2 pneumatic drive rams. The standard single-mix system is equipped with 2 drive syringes and 1 pneumatic drive ram. The operation of each SHU is identical for single mixing stopped-flow experiments.

The flow circuit comprises 2 drive syringes, 2 drive valves, 3 flow tubes, optical cell, stop valve and stop syringe. With the exception of the last two components, the flow circuit is enclosed by a water bath to provide temperature control of the reagents and their reaction.

Reagents are loaded into separate drive syringes from reservoir syringes. The direction of flow into and out of the drive syringe is controlled by a drive valve. The direction of flow is manually selected by the user via the drive valve control knob.

The SHU features a pneumatic drive system that drives the reagents from the drive syringes through two micro volume flow tubes into an optical flow through cell. The optical cell contains an integral T-mixer which under pressure causes highly efficient mixing of the two reagents. This reaction mixture is stopped in the observation chamber of the optical cell.

Each pneumatic drive displaces the current reaction mixture from the observation chamber to the stop syringe via a waste flow tube and subsequently to a waste vessel.

The volume of each drive is accurately controlled via the Auto-Stop mechanism. This mechanism is also responsible for triggering the data acquisition procedure.

The cell housing employs a removable cartridge design in which the optical cell is mounted. This provides the capability for rapidly changing the optical cell according to experimental requirements.

The cell housing features five observation ports that provide user friendly flexibility in measurement of absorbance and fluorescence based signals.

2.4.1.2 Description of a Stopped-Flow Drive

The following sequence of events occurs for each stopped-flow drive:

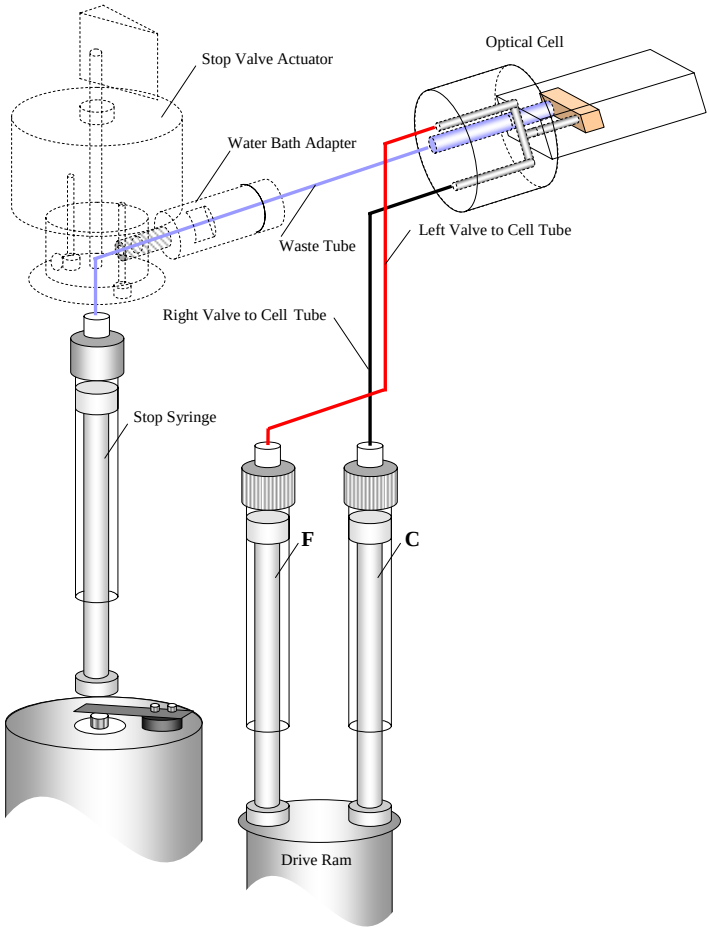
1) The stop syringe is emptied of a set volume of solution by the auto-stop mechanism. This volume equates to the drive volume that will be mixed later in the sequence by the drive ram. There are three steps to this process. Firstly, the stop valve is turned to the *Empty* position. Secondly, the return cylinder is raised to displace a fixed volume of solution from the stop syringe. Finally, the return cylinder returns to its initial position and the stop valve is switched to the *Drive* position.
2) The drive ram fires, pushing the reagents from the two drive syringes through the flow tubes into the optical cell. The optical cell contains a T-mixer that accounts for thorough mixing of the two reagents prior to the observation window.
3) The flow of the mixed solution is stopped at the moment the stop syringe plunger contacts the copper trigger on the Auto-Stop mechanism. I.e. once the selected drive volume has been mixed.
4) A signal is monitored as set-up in the software.

2.4.1.3 Stopped-Flow Circuit

A simplified view of the single mix flow circuit is portrayed in the diagram overleaf. The flow circuit consists of the following key components:-

2 drive valves for loading the reagents into the drive syringes
2 drive syringes for storing the reagents prior to mixing
2 flow tubes for transferring the reagents from the drive syringes to the optical cell
1 optical cell with an integral T-mixer where the reagents are mixed and an observation chamber with two different optical pathlengths.
1 waste flow tube for transferring the mixed reagents from the optical cell to the Auto-Stop unit.
1 stop valve that controls the flow from the optical cell to the stop syringe and from the stop syringe to the waste collection vessel.
1stop syringe that is emptied and filled during the stopped-flow process.

The sequential (double) mixing flow circuit features two additional drive valves and drive syringes plus extra flow tubing. This configuration will be described fully later in this Chapter.


Optical Cell
Stop Valve Actuator
Water Bath Adapter
Waste Tube
Left Valve to Cell Tube
Right Valve to Cell Tube
Stop Syringe
F
C
Drive Ram

2.4.1.4 Drive Syringes

Two 2.5mL drive syringes are fitted to the standard single mixing SHU. The drive syringe is screwed into the base plate of the drive valve and is filled with the reagent solution that is mixed during the stopped-flow drive. The plastic piston is constructed of PEEK to protect against corrosion. The tip of the piston is Teflon. The SHU water bath is fitted with rubber grommets to provide a leak-free seal between the drive syringe and the water bath housing.

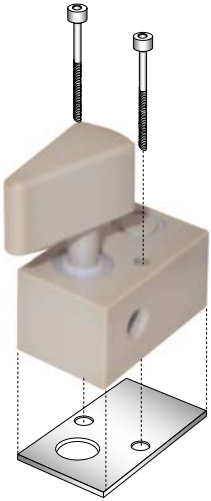
In addition to the standard Kloehn manufactured 2.5mL syringe, use of Hamilton syringes of different volumes may be used to achieve asymmetric mixing ratios. This is described in detail later in this Chapter. However, it is essential that if asymmetric mixing ratios are attempted the drive pressure must be reduced using the external pressure regulator fitted to the pneumatic inlet on the rear of the SHU.

The normal temperature operating range of the drive syringes determines the operating range of the SX20 to 5-50^{o}C. Above 50^{o}C, the adhesive used to bond the syringe will deteriorate. Below 5^{o}C the syringe piston tip does not seal with the glass barrel. A special low temperature syringe is available for work below 10^{o}C.

2.4.1.5 Drive Valves

Two PEEK drive valves are fitted to the standard single mixing sample handling unit. There are left-hand and right-hand versions of the drive valve. The drive valve is fixed in position using a stainless steel base plate and two securing screws. SHUs fitted with the sequential (double) mixing option will be fitted with four drive valves.

The picture to the right shows a right-hand drive valve with its respective base plate and screws. Note that the two O-rings fitted to seal the valve are not shown th



The drive valves control the flow of solution between the drive syringe and either the reservoir syringe or the flow-circuit. The *Load* position is used to control the flow of the drive syringe to and from the reservoir syringes via a female leur fitting. The *Drive* position is used to control the flow from the drive syringe towards the optical cell via a PEEK micro volume flow tube. Each drive valve is controlled manually by turning the control knob 90^{o} between the *Load* and *Drive* positions. The picture below shows the orientation of the valves in both positions.

*Valves in **Drive** Position*

*Valves in **Load** Position*

2.4.1.6 Stop Syringe

A 2.5mL stop syringe is fitted to the SHU. Its purpose is to act as a small reservoir of waste solution from which a volume equal to the drive volume may be displaced prior to the stopped-flow drive. At the time of the drive, the stop syringe is refilled and the action of the plunger striking the trigger indicates that the drive is completed and activates the acquisition of data. The stop syringe is screwed into the lower threaded port of the stop valve and supported in this position by the stop syringe clamp. The plastic piston is constructed of PEEK to protect against corrosion. The tip of the piston is Teflon.

In addition to the standard Kloehn manufactured 2.5mL stop syringe, use of other stop syringes may be used for specific experiments. For rapid kinetic measurements (rate constants in excess of $500s^{-1}$) a smaller volume 1mL Hamilton manufactured stop syringe is recommended for improved data fitting. This is described in detail later in this Chapter.

2.4.1.7 Stop Valve

The stop valve controls the flow of solution from the flow circuit into the stop syringe and from the stop syringe to the waste vessel. The stop valve may be turned manually using a control knob or automatically via the software controlled Auto-Stop mechanism. The *Drive* position is used to control the flow from the flow circuit to the stop syringe. The *Empty* position is used to control the flow from the stop syringe to the waste vessel. The *Drive* and *Empty* positions are shown in the diagrams below.

*Stop Valve in **Drive** Position*

*Stop Valve in **Empty** Position*

The body of the valve is constructed of PEEK with a plastic spindle. The 180^{o} rotation of the spindle provides control of the flow direction. The spindle is retained in the valve body by a stainless steel plate.

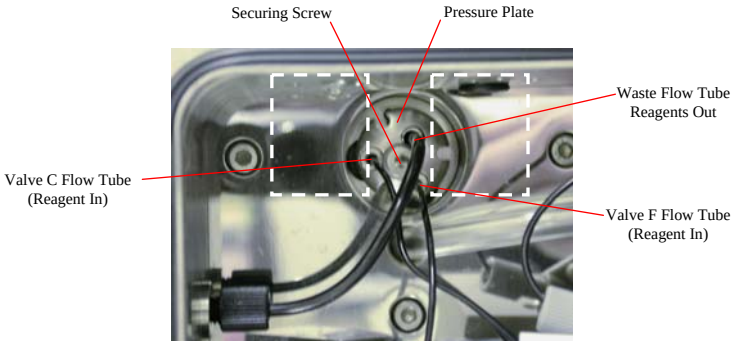
A stainless steel adaptor is screwed into the entrance port of the stop valve to accommodate the PEEK flow tube connected to the optical cell. A polypropylene tube is connected to the exit port to flow to the waste vessel.

2.4.1.8 Flow Tubing

The flow circuit utilises custom-made PEEK (polyetheretherketone) tubing terminated with compression fittings to link the drive valves with the optical cell and the cell with the stop valve. The rigidity and chemical inertness of PEEK make it ideally suited for this purpose. The internal diameter of all the flow tubing before the cell entrance is 1.56mm. The waste tube connected between the cell and stop valve is 3.12mm.

All the flow tubes are connected to the various valves and connectors in the flow circuit using threaded fittings with the exception of those that connect to the optical cell face. These tubes are attached to the cell using a pressure plate, which enables both inlet tubes and the outlet tube to be tightened or loosened simultaneously.

The arrangement of the pressure plate and flow lines is displayed in the picture below. For the purpose of clarity, the drive valves have been removed.



The single mixing SHU flow circuit comprises the following tubes:

Description	Part Number
Left Hand Valve to Cell	PK25R
Right Hand Valve to Cell	PK15R
Cell to Stop Valve	PK20R

The sequential (double) mixing SHU flow circuit comprises the following tubes:

Description	Part Number
Valve A to Pre-Mixer	PK18
Valve B to Pre-Mixer	PK17
Pre-Mixer	
Pre-Mixer to Connector	PK22
Connector/Pre-Mixer to Cell	PK16R
Valve F to Connector	PK19
Valve C to Cell	PK15R
Cell to Stop Valve	PK20R

PEEK is an inert thermoplastic material that is compatible with a wide range of organic solvents, acids and bases making it an ideal choice for the stopped-flow circuit. PEEK also offers high anaerobic performance for oxygen sensitive stopped-flow experiments provided that the flow-circuit has been prepared carefully.

The compatibility of PEEK with various reagents encountered in stopped-flow measurements is outlined in the table below:

Reagent	Compatibility
1,1,1 Trichloroethane	A
1,2 Dichloroethane	B
Carbon Tetrachloride	A
Chloroform	A
Dichloromethane	B
Dichlorobenzene	A
Ethylene Dichloride	A
Trichloroethylene	A
Aliphatic Esters	A
Butyl Acetate	A
Ethyl Acetate	A
Diethylether	A
Dioxane	A
Petroleum Ether	A
Tetrahydrofuran	A
Benzene	A
Toluene	A
Xylene	A
Hexane	A
Phenol (dilute)	A
Phenol (conc)	C
Dimethylsulphoxide	B
Diphenylsulphone	B
Acetonitrile	A

Reagent	Compatibility
Urea	A
Ammonium Chloride	A
Calcium Salts	A
Copper Salts	A
Iron(II) Chloride	B
Iron(III) Salts	A
Manganese Salts	A
Magnesium Salts	A
Nickel Salts	A
Potassium Salts	A
Silver Nitrate	A
Sodium Salts	A
Tin(II) Chloride	A
Sulphites	A
Hydrogen Peroxide	A
Bleach	A
Soap Solution	A
Sodium Hydroxide	A
Nitric Acid (Conc)	B
Sulphuric Acid (Con)	B
Hydrochloric Acid	A

A – No attack – no or little adsorption
B – Slight attack
C – Severe attack

2.4.1.9 Interchangeable Cell Cartridge System

The SX20 sample handling unit features a novel cell cartridge system that facilitates cartridge mounted optical cells to be interchanged with a minimum of work. The diagram below shows a cell cartridge removed from the cell block.

Procedure for changing the optical cell

The cell currently fitted in the cell block of the sample handling unit should be removed according to the following procedure:

1) Remove the light guide, fluorescence detector and fluorescence cut-off filter holder from the cell block.
2) Drain the contents of the thermostat bath housing.
3) Loosen the pressure plate that seals the three flow tubes against the face of the cell cartridge. (No more than one turn is sufficient).
4) Undo the four fixing screws that secure the cell cartridge to the cell block.
5) Carefully withdraw the cell cartridge to the rear.

The refitting procedure is the reverse of the removal:

1) Ensure that the O-ring seals are present.
2) Insert the cell cartridge into the cell block so that the bevelled corner of the cartridge is aligned with that on top of the cell block.
3) Secure the cartridge in position with the four captive fixing screws.
4) Tighten the tubing pressure plate screw to ensure a good seal between the three flow tubes and the cell.
5) Replace the light guide and detectors.

6) Flush the flow circuit with distilled water until all bubbles have been expelled.
7) Reattach the thermostat bath cover.

Whenever any component of the flow circuit has been altered, upon reassembly the user is recommended to check the flow circuit for leak-free operation.

Checking the flow circuit for leaks

Leaks in the flow circuit will result in collection of unreliable data and loss of sample. The flow circuit may be checked for leaks using the following procedure:-

1) Fill the flow circuit with water or solvent.
2) In the software, set-up a stopped-flow drive for 10s with the drive pressure held.
3) During the 10s drive, closely observe the tips of the drive syringe pistons.
4) If the pistons do not creep upwards the flow circuit is leak-free.

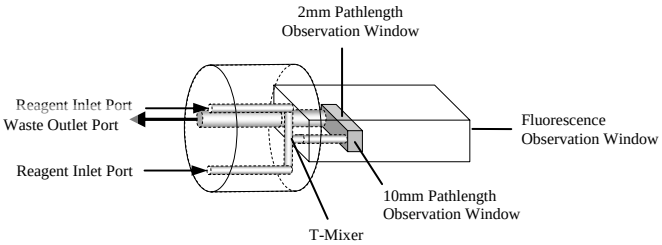
The flow circuit is full when the stop syringe contacts the auto-stop trigger initiating data acquisition. At this moment the drive syringes should not be able to push more solution from the drive syringes and the pistons should not creep upwards.

If the flow circuit leaks, the pressure held on the drive syringes over 10s will push the solution from the drive syringes out of the flow circuit at the location of the leak. In this case the pistons will appear to creep upwards during the 10s period.

A more detailed description of identifying leaks and their repair is given in the maintenance section of this chapter.

5.1.10 Optical Cells

All optical cells available for use on the SX20 are construction from quartz and feature an integral flow circuit and T-mixer located in the quartz. The dimensions of the optical windows of the cell provide the user with different optical pathlengths and dead times. The diagram below shows the internal design of the 20µL optical cell:



Cell Pathlengths and Dead Times

The table below lists the pathlengths and approximate stopped-flow dead times of the optical cells available from Applied Photophysics.

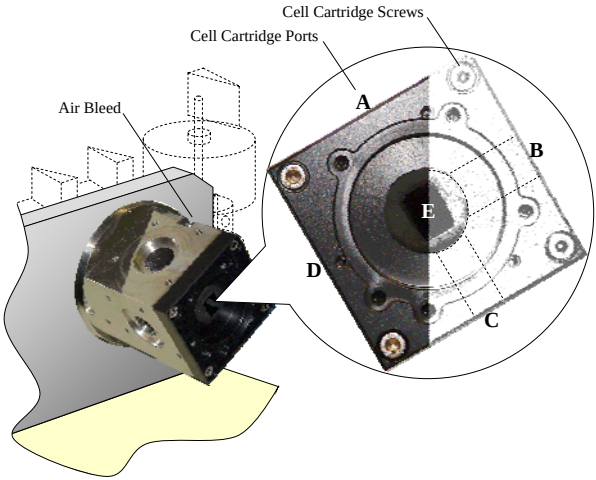
Cell Volume	Pathlengths	Uses	Dead Time
20µL	10mm +2mm	Standard SF	1ms
5µL	5mm + 1mm	Rapid SF	500µs

All SX20 systems are supplied with the standard 20µL optical cell. This cell is the most sensitive for emission based measurements and has a dead time of approximately 1ms. The 5µL cell provides extra pathlength options and a shorter dead time (500µs) however the fluorescence sensitivity is lower than the standard cell owing to the smaller fluorescence window in the 5µL cell.

Note that the 5µL cell must be operated with the 1mL stop syringe provided with the cell. Rapid kinetic measurement will be described in detail later in this chapter of the User Manual.

2.4.1.11 Absorbance Pathlength Selection

The optical cells feature a long and short pathlength (10mm and 2mm for the standard 20µL cell). The pathlength is selected according to the positioning of the light guide and absorbance detector on the cell block. The diagram shows the rear of the cell block and the configuration of the light guide and detector to select each pathlength.



The long pathlength is between ports C and A. The short pathlength is between ports D and B. The optical pathlength is selected by moving the absorbance PMT detector (A or B ports) and light guide (C or D ports) to the respective ports on the cell block.

The absorbance pathlength also applies to using the optional photodiode array detector. Further information on use of the PDA is provided later in this chapter.

Fluorescence Excitation Pathlength Selection

The fluorescence detector mounting (port E) is at the rear of the cell block and the emission PMT detector should be mounted using the rubber light seal, finger screws and nylon washers provided. When measuring fluorescence the excitation light guide should be connected to the D port to give the best illumination and lowest inner filter effect.

2.4.1.12 The Auto-Stop Mechanism

The Auto-Stop mechanism is mounted on the front left hand side of the Sample Handling Unit. It has two main functions:-

1) To control the volume of each stopped-flow drive.
2) To trigger data acquisition at the moment the flow is stopped.

The Auto-Stop mechanism, shown below comprises a number of important components:-

Auto-Stop Actuator - This provides automated stop valve control via the software. It switches between the *Drive* and *Empty* positions driven by compressed gas (8bar). The plastic control knob provides an option to control the stop valve manually.

Stop Valve - This controls the flow of reagents into the stop syringe (*Drive* position) and out of the stop syringe to the waste vessel (*Empty* position).

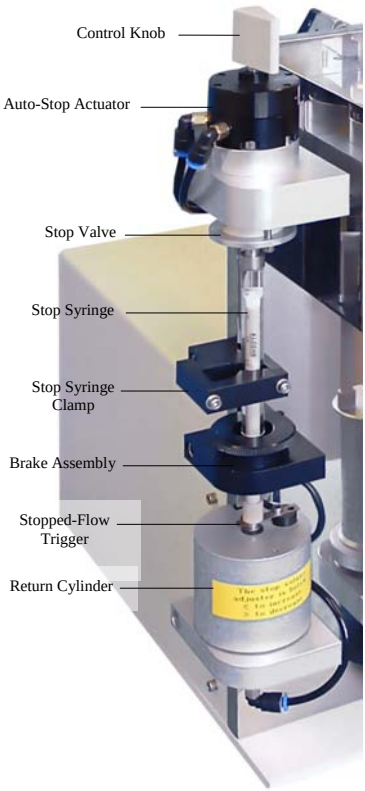
Stop Syringe - The emptying of this syringe controls the drive volume. A number of syringe options are available.

Stop Syringe Clamp - This provides support for the stop syringe.

Brake Assembly - This is required only for sequential (double) mixing experiments.

Stopped-Flow Trigger - This completes a circuit once contacted by the piston of the stop syringe to start the data acquisition.

Return Cylinder - This upward movement of the cylinder empties the stop syringe. It also contains the adjuster for setting the drive volume. The Return Cylinder is pneumatically driven by compressed gas.



Setting the Drive Volume

The volume of a stopped-flow drive is controlled using the adjuster screw on the return cylinder. This adjuster sets the vertical limit of travel for the return cylinder. The zero drive volume is set by turning the screw clockwise to the limit of its travel. The volume is increased by releasing the screw a set number of turns.

Using the standard 2.5mL stop syringe, one full turn of the adjuster screw equates to a total drive volume of approximately 40µL.

The exact drive volume required for a stopped-flow experiment will depend on a number of factors including the type of measurement, the acquisition time and physical properties of the reagents. Recommended drive volumes are provided in the relevant sections of this chapter that discuss

Software Control of the Auto-Stop Mechanism

The stop syringe is emptied by clicking on the Empty button in the Pro-Data software Control Panel. This action completes the empty cycle of the Auto-Stop mechanism. This function is useful for priming the flow-circuit during the experimental set-up routine to maximise sample economy.

The software provides control of three solenoid valves that open and close to apply high pressure to the actuator and return cylinder via three plastic tubes labelled A, B and C. A rotates the stop valve to the *Empty* position, B raises the return cylinder to empty the stop syringe by a set volume and C rotates the stop valve back to the *Drive* position. The timing of these operations is critical and is controlled via the Waste Timings function in the Pro-Data software Control Panel. Under normal circumstances the user will not need to alter these timings

Manual Control of the Auto-Stop Mechanism

The Auto-Stop mechanism may be manually operated to empty the stop syringe. This is particularly useful for cleaning the flow circuit before and after measurements. To empty the stop syringe, the following procedure should be used:

1) Turn the control knob clockwise 180^{o} to the rear. This is the *Empty* position.
2) Grip the lower part of the Stop Syringe plunger and push upward expelling the contents of the syringe to the waste vessel.
3) Return the control knob anti-clockwise 180^{o} to the front. This is the *Drive* position.

Stop Syringe Brake Mechanism

The brake mechanism, (see Auto-Stop picture), is provided to improve the results obtained when performing sequential (double) mixing experiments. It is used to provide friction on the stop syringe plunger and hence prevent over-run after the first drive. This would result in cavitation in the flow line and inconsistent results. The friction is increased by tightening the mechanism, decreased by loosening it. It must be loose for single mixing stopped-flow so that it does not impair the dead time of the instrument, but tightened up, as necessary, when performing sequential mix experiments.

Note that the sequential mixing brake assembly is only fitted to SHUs equipped with the sequential (double) mixing option (SQ.1)

2.4.1.13 Pneumatic Drive and Drive Rams

The SX20 Sample Handling Unit features a pneumatic drive system. The drive ram acts as the interface between the pneumatic drive system and the flow circuit. To satisfy the differing needs of the single mixing stopped-flow and sequential (double) mixing stopped-flow techniques, three drive rams are supplied on the full sequential-flow machine. On the single-mixing instrument, only one drive ram is supplied. The picture below shows the three drive rams compatible with the SX20.

Single Mix Drive Ram

Flush and Pre-Mix Drive Rams

Sequential mixing experiments require great care in setting up the volumes for the first and second mixing drives. To assist the user in setting up the correct drive volumes, an electronic transducer is fitted in each drive ram mounting platform. Movement of the transducer is reported in the software and may be used to calculate the drive volume. The limited travel of the transducer necessitates the use of special sequential mix drive rams with a similar travel limit. In addition, the pre-mix drive ram contains a self-stop mechanism that allows the volume of the pre-mix drive to be set via this mechanism. The flush ram is not fitted with this mechanism and the corresponding drive volume is set via the auto-stop mechanism.

Further information on the use of the transducer profile information to set up sequential mixing experiments is provided later in this chapter.

2.4.1.14 Temperature Control

The sample handling unit is fitted with a water bath to provide temperature control of stopped-flow experiments. A circulator unit may be connected to pump thermostatic fluid into the water bath housing surrounding the drive syringes, drive valves and flow tubing. The thermostatic fluid also fills the cell block ensuring temperature control of the entire flow circuit.

A thermocouple located in the PEEK housing reports an accurate temperature measurement inside the water bath to the software. This temperature is recorded with each stopped-flow drive.

Circulator Unit Connections

A circulator unit is connected to the SHU water bath via 8mm tubing. The SHU is fitted with quick fit Legris-type fittings to allow the circulator to be connected and disconnected rapidly without the need to drain the water bath. The rear port is the outlet and the front port is the inlet.

For circulator units equipped with an external temperature probe to compensate for temperature loss between the circulator and the external water bath, a probe port is provided on the right side of the

Software Controlled Circulator Units

Certain circulator units may be connected to the serial port of the workstation PC via a RS232 cable to provide temperature control in the SX20 control software. The following circulator units may be linked to the workstation PC:

Neslab RTE 200
Fisher Scientific 3016

For further information on circulator unit compatibility please contact the Technical Support Team at Applied Photophysics.

Recommended Thermostatic Fluids

Applied Photophysics recommend the addition of an anti bacterial agent and antifreeze to distilled water as a suitable thermostatic fluid.

Draining the Water Bath

The water bath can be drained according to the following procedure:

1) Switch off the circulator unit.
2) Loosen the bleed screw on top of the cell block using a 2.5mm hexagonal wrench.
3) Allow the water bath to drain.
4) Tighten the bleed screw on top of the cell block.

2.4.2 Absorbance Detector

2.4.2.1 Description

The SX20 is fitted with a side-window 9-stage photomultiplier tube for absorbance detection measurements. The standard detector features a Hamamatsu R928 PMT capable of operation in the wavelength range 185-900nm.

2.4.2.2 Basic Set-Up and Electrical Connections

The detector is mounted to one of the two upper ports on the cell block using two mounting screws. The detector position must always be opposite that of the light-guide (fitted to one of the lower ports) and this configuration determines which optical pathlength of the cell is selected for the measurement. See the section describing the cell block and optical cells for further information on available pathlengths.

Absorbance measurements normally employ the 10mm pathlength of the standard 20µL cell (or 5mm pathlength of the 5µL cell). These require the detector to be mounted to the upper right port and the light-guide to the lower left port of the cell block. Remember to blank off any unused ports.

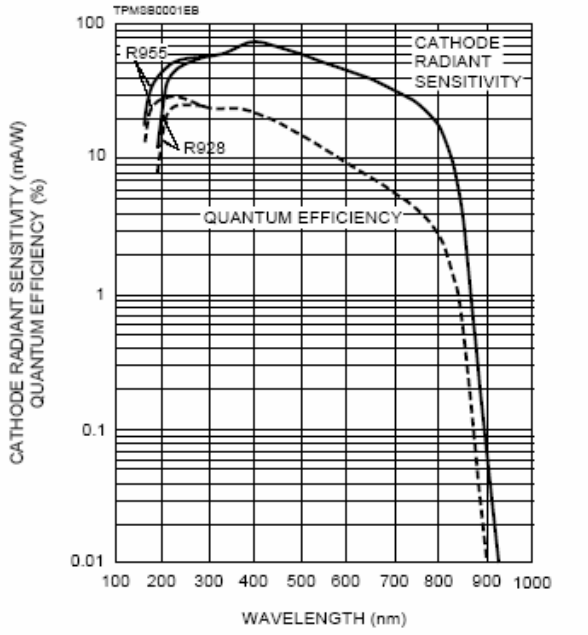
Caution! Before switching the detector between ports, it is essential that any high voltage applied to the detector is switched off via the software to prevent damage to the PMT.

For SX20 systems fitted with a single detection channel, there will be one cable connected to the PMT Module of the Electronics Unit. When switching between absorbance and fluorescence detection, the cable must be connected to the appropriate detector.

For SX20 systems fitted with dual detection channels, there will permanently be two detector cables connected to the PMT Module of the Electronics Unit. The absorbance detector is connected to Channel 1 and the fluorescence detector is connected to Channel 2.

2.4.2.3 PMT Characteristics

The response characteristics of the Hamamatsu R928 PMT are displayed in the diagram below:



2.4.3 Fluorescence Detector

2.4.3.1 Description

The SX20 is fitted with an end-window 11-stage photomultiplier tube for emission detection measurements. The standard detector features a Hamamatsu R6095 PMT capable of operation in the wavelength range 300-650nm. In addition to the standard PMT, Applied Photophysics offer alternative PMTs for applications requiring different detection characteristics.

2.4.3.2 Basic Set-Up and Electrical Connections

The detector is mounted to the fluorescence port at the rear of the cell block using 3 finger screws. When fitting the detector, ensure that the detector light seal and the seals in the cell block are present to prevent detection of stray light.

Fluorescence measurements are normally carried out using the 2mm pathlength of the standard 20µL cell (or 5mm pathlength of the 5µL cell). These are accessed by fitting the excitation light-guide to the lower right port on the cell block. Remember to blank off any unused ports.

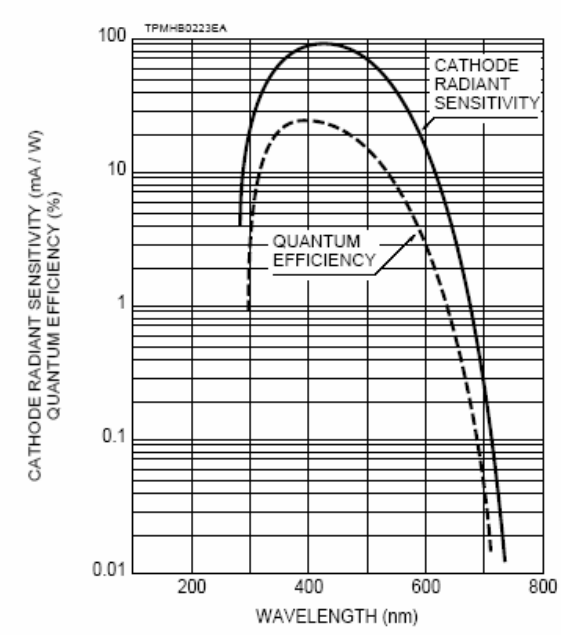
Caution! Before removing the detector from the cell block, it is essential that any high voltage applied to the detector is switched off via the software to prevent damage to the PMT.

For SX20 systems fitted with a single detection channel, there will be one cable connected to the PMT Module of the Electronics Unit. When switching between absorbance and fluorescence detection, the cable must be connected to the appropriate detector.

For SX20 systems fitted with dual detection channels, there will permanently be two detector cables connected to the PMT Module of the Electronics Unit. The absorbance detector is connected to Channel 1 and the fluorescence detector is connected to Channel 2.

2.4.3.3 PMT Characteristics

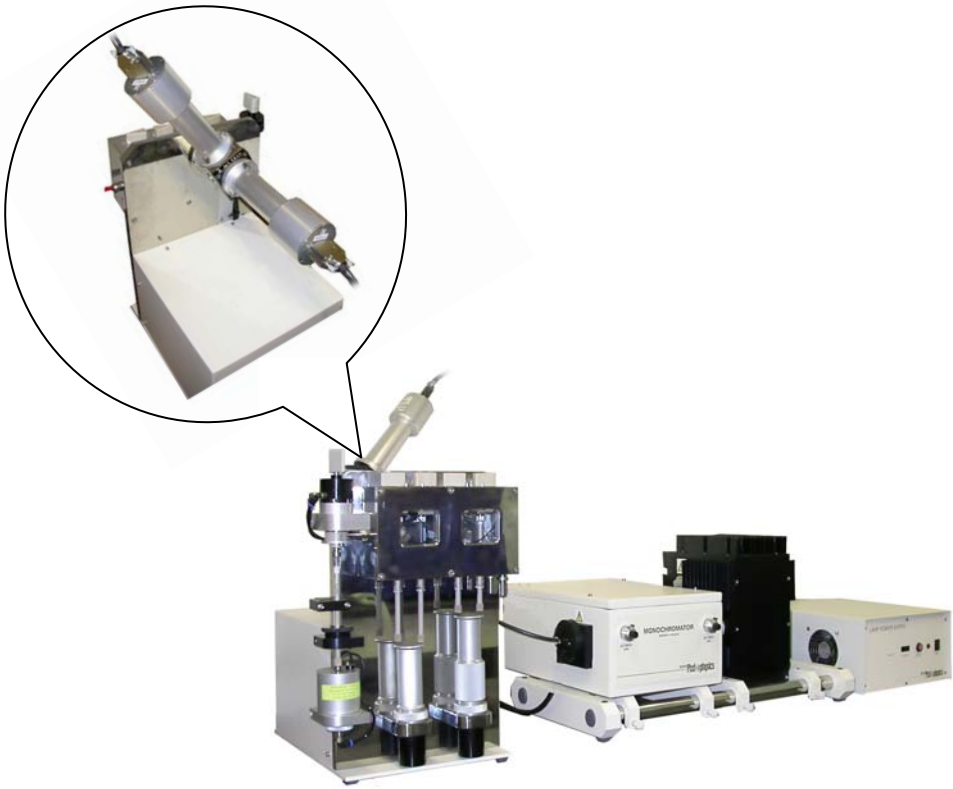
The response characteristics of the Hamamatsu R6095 PMT are displayed in the chart below:



2.4.3.4 Dual Channel Fluorescence Set-Up

For SX20 systems fitted with the dual channel fluorescence detection or fluorescence polarisation options, the second fluorescence detector must be connected to Channel 1 of the Electronics Unit PMT Module. This channel is normally used for absorbance measurements and the fluorescence detector is best connected by disconnecting the detector cable from the absorbance detector and connecting to the second fluorescence detector.

For dual channel fluorescence detection, the second fluorescence detector should be mounted on the upper right port of the cell block according to the picture below using the twist-lock fittings supplied. The male fitting is connected to the cell block using 4 countersunk screws ensuring that the light sealing O-ring is fitted. The female fitting is connected to the end of the fluorescence detector using 3 screws.



2.4.4 Monochromator

2.4.4.1 Description

The high precision automated monochromator contains a 250nm holographic diffraction grating that provides a useful operating wavelength range of 190 to beyond 850nm (limited by PMT detector range). The monochromator is accurately pre-aligned and calibrated using a He-Ne laser and mercury emission lines.

The wavelength of monochromatic light is selected by rotation of the grating using a stepper motor housed within the monochromator unit. This motor is controlled via Control Module 1 in the Electronics Unit. This allows the monochromator wavelength to be set via the SX20 control software.

The monochromator entrance and exit slits are manually adjustable using the dials on the front of the monochromator. The dials are calibrated in millimetres and are mechanically linked to a pair of slit blades on the entrance and exit. A 1mm monochromator slit opening is equivalent to a wavelength bandwidth of 4.65nm. The slits provide a bandwidth range from below 0.5nm up to 37 nm.

2.4.4.2 Basic Set-Up and Electrical Connections

The monochromator is located on the optical rail to the left of the lamp housing. It is essential that the lamp housing and monochromator are correctly coupled together. The two units must be pushed together so that the exit port of the lamp locates firmly with the entrance port of the monochromator. To prevent vibration, both units should be locked in position on the optical rail using the four plastic locking screws on each unit.

The monochromator control cable is connected to Port 1 of Control Module 1 in the Electronics Unit. For systems with two monochromators, Mono 1 must be connected to the Drive 1 port and Mono 2 must be connected to the Drive 2 port.

2.4.4.3 Selecting a Suitable Monochromator Slit Width (Band Pass)

The monochromator slit width setting is an important variable in the set-up of a stopped-flow experiment. Typically these values lie in the range 0.25 to 2mm. A suitable value will depend on numerous factors, the most important factors being the type of signal being measured and the stability of the reagents to photochemical degradation (photo-bleaching). The entrance and exit slit widths are normally set to identical values.

A suitable setting for absorbance experiments is 0.5mm.

Fluorescence experiments tend to be more sensitive and require higher light throughput using wider slit width settings. 1mm and 2mm settings are commonly used.

It should be noted that samples which are susceptible to photochemical reaction should be investigated with a narrower slit setting to reduce the possibility of photo-bleaching.

2.4.4.4 Band Pass Calculation

The wavelength bandpass is calculated by multiplying the slit width readings (in mm) by a factor of 4.65. Bandpass below 2 nm are most accurately set using feeler gauges. The final bandpass adjustment should always be set by moving the dial in a clockwise direction. This procedure will give maximum accuracy and reproducibility.

2.4.4.5 Dual Monochromator Operation

Systems equipped with two monochromators are labelled *Mono 1* and *Mono 2*. *Mono 1* is always connected to the lamp housing on the lower rail. *Mono 2* may either be positioned on the upper rail in the emission configuration or on the lower rail to the left of *Mono 1* in the UV absorbance configuration according to the experimental requirement.

2.4.4.6 Photodiode Array Operation

When the SX20 is used with the optional photodiode array accessory (PDA.1), the monochromator is automatically set to the zero order position by the software. This allows white light from the lamp source to pass directly through the monochromator to the optical cell. The user must adjust the entrance and exit slit widths to maximise the signal reaching the photodiode array unit. A detailed description of photodiode array operation is provided later in this chapter of the user manual.

2.4.4.7 Absorbance Measurements in the near IR (>650nm)

It is essential that absorbance measurements collected at wavelengths greater than 650nm use an appropriate filter to eliminate second order stray light from detection. Second order stray light is a phenomenon common to all monochromators fitted with diffraction gratings. In addition to monochromatic light of the required wavelength, a small proportion of light with half this wavelength is also generated.

All SX20 systems are supplied with a 645nm cut-off filter that should be positioned between the optical cell and the absorbance detector when measuring absorbance in the near IR region (>650nm).

2.4.4.8 Maintenance

The monochromator should not require any maintenance from the user. Under no circumstances should the user attempt to recalibrate the monochromator.

2.4.5 150W Lamp Housing

2.4.5.1 Description

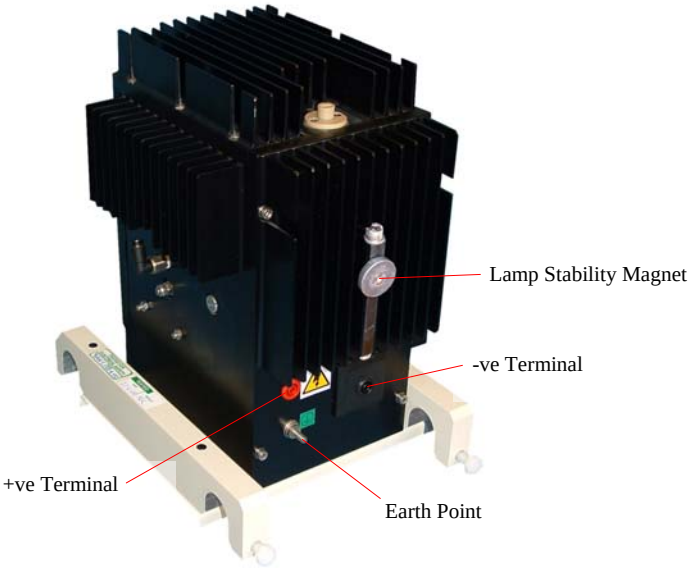
The arc lamp source uses a 150W gap shortened xenon bulb and is ignited and powered by the lamp power supply unit. Three different lamps may be fitted to the housing to accommodate experimental requirements.

A manually operated shutter is located on the exit port of the lamp housing to control the passage of light to the monochromator. The shutter is open when the shutter is pushed to the rear. The shutter is closed when pulled to the front.

2.4.5.2 Basic Set-Up and Electrical Connections

The 150W lamp housing is mounted on the right hand side of the optical rail. A magnet assembly is sometimes attached to the rear of the housing to provide greater stability of the xenon arc lamp.

The lamp housing is fitted with two input terminals (black negative and red positive) that are connected to the lamp power supply unit. The earth post should be connected to the earth post on the optical rail using the braided cable supplied.



2.4.5.3 Lamp Stability Magnet

The SX20 lamp housing is sometimes fitted with an adjustable magnet assembly that stabilises the xenon arc. The magnet is attached to the rear of the lamp housing in the orientation that provides the greatest stability.

2.4.5.4 Lamp Types

There are three types of 150W arc lamp approved for use in the SX20 lamp housing. The choice of lamp will depend on individual experimental requirements. Systems are normally supplied fitted with a 150W **ozone-free xenon lamp** unless requested otherwise. This lamp has excellent long term stability and a spectral range of 250-1000nm.

For studies in the far UV region, a 150W **ozone-producing xenon lamp** is recommended, with a spectral range of 200-1000nm. Operating with an ozone producing lamp requires the lamp housing to be purged with an inert gas such as nitrogen. The purging procedure is described later in this section.

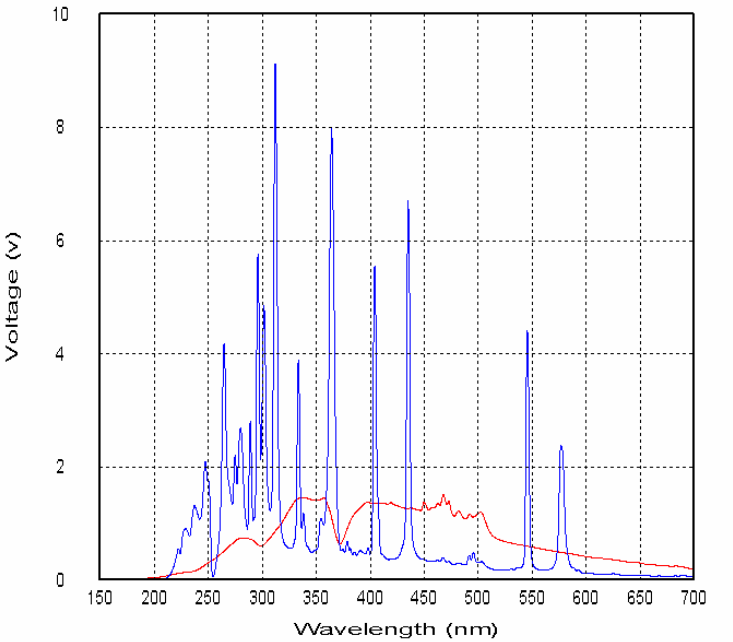
For fluorescence-based measurements, a 150W **mercury-xenon lamp** is available that offers significantly higher output at particular wavelengths. The lamp emission profile relative to the standard 150W source is shown overleaf and is accompanied by a table listing the most significant mercury emission lines. Fluorescence excitation at these wavelengths provides an improved signal to noise level in fluorescence data compared to a standard 150W xenon lamp.

Additional lamps can be supplied mounted on an extra lamp housing unit back-plate and storage case. This allows for more rapid lamp changeovers and eliminates the need for lamp alignment following the swap.

The table below lists the three lamps approved for use with the SX20 system:

Lamp	Manufacturer	Uses
150W Xe (O_3 free)	Osram XBO 150W/CR OFR	general SF
150W Xe (O_3 producing)	Osram XBO 150W/4	Far UV region SF
150W Hg-Xe	Hamamatsu L2482	Sensitive fluorescence SF

The output profiles for the xenon lamp (red) and mercury xenon lamp (blue) are displayed in the diagram below:



The principle emission lines of the mercury-xenon lamp are listed in the table below:

Wavelength	Intensity	Wavelength	Intensity
222	6.0%	302	52.0%
230	9.8%	313	100%
240	14.2%	334	37.1%
250	19.2%	365	89.5%
265	45.6%	405	60.8%
277	23.9%	436	72.8%
283	28.8%	546	47.9%
290	30.7%	577	25.4%
297	63.1%		

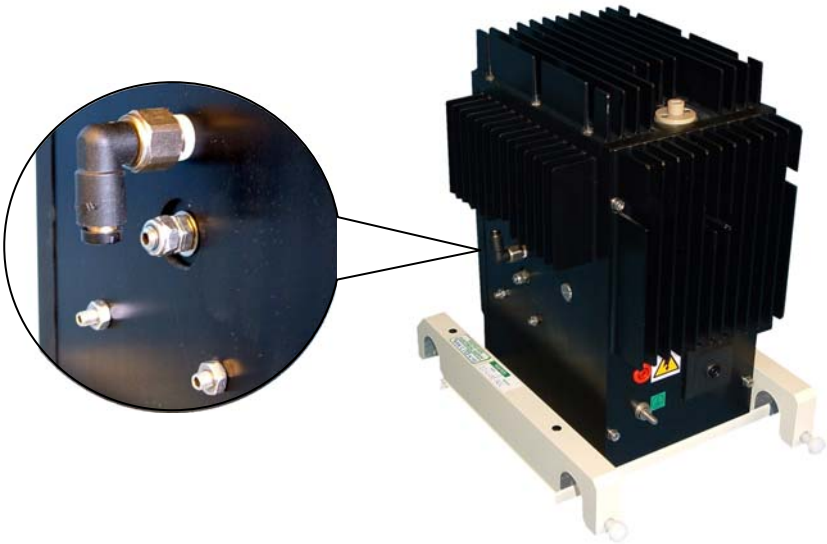
2.4.5.5 Purging the Lamp Housing (Ozone-Producing Lamp Only)

Caution! Ozone inhalation is harmful to health.

This procedure applies only to lamp housings fitted with an ozone-producing xenon lamp. Ozone is produced by the reaction of oxygen in the atmosphere with UV light. Inhalation of ozone is harmful to health and will rapidly degrade seals and optical surfaces in the lamp housing and monochromator. Purging the lamp housing with an inert gas such as nitrogen will ensure there is no build-up of ozone in the lamp housing. A fitting is provided on the right hand side of the lamp housing to connect a purging line from the main stopped-flow drive supply.

Connection of the Lamp Housing Purging Accessory

If nitrogen is being used as the main drive gas supply then the elbow on the h-shaped tubing arrangement used to connect the gas supply to the back of the sample handling unit can be replaced with an additional flow regulator assembly. The tubing on the outlet of the flow regulator should be connected to the purge inlet on the right hand side of the lamp housing as shown in the picture below.



Purging Procedure

Before igniting the lamp, the flow regulator should be opened to allow a minimal steady flow of nitrogen through the lamp housing. This flow should be continued for at least 15 minutes prior to igniting the lamp. This will ensure that all the air in the lamp housing is replaced with nitrogen. The nitrogen flow **must** be maintained when the lamp is running.

2.4.5.6 Lamp Alignment Procedure

The lamp alignment should be checked frequently to ensure optimum performance of the SX20 system. Any misalignment will result in excess noise, due to both a lower light level and, more importantly, increased vibrational noise. The alignment procedure involves adjusting the horizontal and vertical position of the lamp in the lamp housing to optimize the signal monitored in the live display window of the software.

Caution! To prevent risk of electrical shock and damage to the instrument, the alignment procedure must only be performed using the insulated 4mm hexagonal wrench supplied with the SX20 system.

1) Remove the magnet assembly from the rear of the lamp housing. Note the orientation of the magnet if fitted.
2) Loosen the lamp alignment mechanism locking screw on the rear of the lamp housing. Generally no more than one quarter turn should be necessary.
3) Remove the two plastic plugs covering the alignment screws.
4) The sample handling unit should be configured in the absorbance mode. Set the monochromator to a suitable wavelength for aligning the lamp. Applied Photophysics recommend a value of 350nm with monochromator slits set to 0.5mm.
5) Click the Reference button in the baseline panel of the control software to set the absorbance baseline.
6) Adjust the horizontal alignment setting by turning the hexagonal wrench until the signal reaches an optimum level (i.e. minimum absorbance).
7) Adjust the vertical alignment setting by turning the hexagonal wrench until the signal reaches an optimum level (i.e. minimum absorbance).
8) Repeat the horizontal and vertical alignment steps until the optimum signal is reached.
9) Retighten the lamp alignment mechanism locking screw and refit the magnet assembly to the rear of the lamp housing.
10) Replace the two plastic plugs covering the alignment screws.

2.4.5.7 Lamp Lifetime

The 150W arc lamps are generally rated for 1000 operational hours. The life time of a lamp often exceeds 1000 hours depending on the type of work it is used for. Symptoms of an aging lamp include difficulty to ignite, poor lamp stability and reduced output (particularly in the UV region). When the lamp starts to exhibit these symptoms it should be replaced.

Note that lamps operated in excess of 1000 hours are more susceptible to break or explode causing damage to the interior of the lamp housing.

2.4.5.8 Lamp Stability Test

The stability of a lamp is a performance measure that will determine the quality of the data that can collected on the SX20. Lamp stability can be measured according to the following procedure:

1) The sample handling unit should be configured in the absorbance mode with a 2mm optical pathlength.
2) Set the monochromator to 350nm with entrance and exit slits set to 0.5mm.
3) Click the Reference button to apply a high voltage to the detector.
4) Acquire data over a 10s period.

The maximum peak-to-peak noise on the signal during the acquisition period should not exceed 0.001AU for a new system. As systems and lamps age, the stability level decreases. It is recommended that the lamp alignment should be checked if the lamp stability has deteriorated significantly. If the stability exceeds 0.003AU the lamp should be considered for replacement.

2.4.6 Lamp Power Supply Unit

2.4.6.1 Description

The lamp power supply unit (PSU) is a purpose-designed unit for igniting and running the 150W xenon arc lamp. The unit contains a safe-start igniter that is harmless to surrounding electronic equipment. The igniter delivers a high voltage pulse to strike the arc lamp and once ignited supplies a stable running voltage to the lamp.

2.4.6.2 Basic Set-Up and Electrical Connections

The 150W xenon lamp power supply unit is generally located to the right of the optical rail. Suitable clearance to the rear and left hand side should be allowed for the ventilation fan to operate efficiently.

The PSU has one mains inlet socket and two sockets (red and black) for connecting the cables to the lamp housing. The red cable is the positive. The black cable is the negative and also carries the 13000V ignition pulse to start the lamp, hence the heavy insulation. The earth post should be connected to the earth post on the optical rail using the braided cable supplied.

The Lamp Cont socket on the rear of the PSU is currently redundant.

2.4.6.3 Operation

The mains electricity supply to the power supply unit is controlled by the black rocker switch on the front panel of the PSU. The power is switched on by moving the switch to the on (I) position. The red LED indicates that power is present.

To ignite the lamp, press the red Start button on the front panel of the PSU. If ignition is successful the digital timer display will be activated. Xenon arc lamps typically require 30 minutes to warm up and stabilise.

The digital timer displays the number of hours that the lamp has been running. Adjacent to the timer is a recessed button to reset the timer when the lamp is replaced.

The lamp is switched off by moving the rocker switch to the off (O) position.

2.4.6.4 Use of Xenon and Mercury-Xenon Lamps

The lamp PSU is designed to supply a running current appropriate for a 150W Xenon lamp and a 150W Mercury-Xenon Lamp. A switch on the rear panel labelled 8.5A and 7.5A provides a simple way to control the running current for these lamps. When using a xenon lamp, ensure the switch is set to the 8.5A position. When using a mercury-xenon lamp set the switch to the 7.5A position. An incorrect setting will result in increased noise visible on data traces.

2.4.6.5 Maintenance

The lamp PSU contains no user serviceable components and should not require any maintenance. In case of a damaged fuse, the 3.15A mains fuse holder is located above the mains inlet socket.

2.4.6.6 Electrical Characteristics

The characteristics of the lamp power supply and igniter unit are as follows: -

Supply voltage	*85-264V*
Supply frequency	*47-63Hz*
Power rating	*250VA*
Fuse	*3.15A*

2.4.7 Electronics Unit

2.4.7.1 Description

The Electronics Unit provides automated control of the sample handling unit and monochromator wavelength setting plus signal acquisition from the photomultiplier tube detectors and data processing.

The modular design of the SX20 electronics means only required features need be installed and any faults that develop are localised and can be easily repaired by substitution of the appropriate module. All modules have a built in self-test capability which communicates any operational problem to the user.

The rear view of the SX20 Electronics Unit is shown in the picture below:

The full functionality and connections of the Electronics Unit modules are described in order from left to right:

Mains Inlet – The Electronics Unit mains electricity supply inlet is connected to the mains distribution board. The universal mains

12V Power Supply Module – This provides 12V power to all electronics modules of the SX20 except the Communications Module. A red LED indicates that the module is operational. Two sockets are provided for monitoring the output level of the module.

5V Power Supply Module - This provides 5V power to all electronics modules of the SX20. A red LED indicates that the module is operational. Two sockets are provided for monitoring the output level of the module.

Communications Module – This is the interface of the electronics and the fibre-optic link to the computer, it also provides several extra inputs for analogue temperature probes and a general-purpose digital I/O.

The module is connected to the PCI card housed in the workstation PC via a fibre-optic communication cable. The Temp Inputs port is currently redundant on the SX20.

The function of the red Tx and Rx LEDs are identical to those on the front panel of the Electronics Unit. These LEDs flash to indicate that information is being transmitted and received. The green Status LED reports a fault on the module electronics. This is constantly illuminated if the module passes the self test routine and flashes if the test is failed.

Control Module 1 – This provides stepper motor control used to drive the monochromators.

For systems with one monochromator, the monochromator must be connected to the Drive 1 port. For SX20 systems fitted with the SEM.1 or AM.1 options, (i.e. two monochromators), Mono 1 must be connected to the Drive 1 port and Mono 2 must be connected to the Drive 2 port.

The green Status LED below each port indicates the operational status of the respective Drive. The LED is constantly illuminated if the Drive passes the self test routine and flashes if the test is failed.

KSHU Module – This provides control of the sample handling unit pneumatics. It also manages inputs from the drive ram transducers and temperature probe.

The sample handling unit is connected via a black cable fitted with a 44-pin D-connector. The Lamp Cont port is currently redundant on the SX20 system.

The green Status LED indicates the operational status of the module electronics. This is constantly illuminated if the module passes the self test routine and flashes if the test is failed. The red TRG LED indicates triggering of the Auto-Stop of the sample handling unit.

PMT Module – This controls the photomultiplier tube detector high voltage and signal measurement. There are single and double channel versions that allow for connection and measurement on one and two channels respectively.

For systems with one data acquisition channel, the detector cable is connected to the Channel 1 port. For systems fitted with two data acquisition channels, the absorbance detector is connected to Channel 1 of the module. The fluorescence detector is connected to Channel 2.

The green Status LED below each port indicates the operational status of the Channel. The LED is constantly illuminated if the Channel passes the self test routine and flashes if the test is failed.

PDA Module (only fitted on systems equipped with the optional photodiode array detector) – This controls all communication with the externally mounted PDA accessory.

The PDA unit is connected to the module via a black cable fitted with a 26-pin D-connector.

The three yellow LEDs labelled 1024, 512 and 256 indicate the type of diode array that is connected to the module. The diode labelled 256 should be illuminated when a PDA is attached as the currently supplied units are 256 element diode arrays. The green Status LED reports a fault on the module electronics. This is constantly illuminated if the module passes the self test routine and flashes if the test is failed.

Two vacant slots in the electronics rack are available for future instrument expansion.

2.4.7.2 Operation

Refer to the picture below for aspects relevant to operation of the Electronics Unit:

The mains electricity supply to the Electronics Unit is controlled by the black rocker switch on the front panel of the unit. The power is switched on by moving the switch to the on (I) position. The green LED labelled Status indicates that power is present. This LED should be constantly illuminated.

When the Electronics Unit is first switched on, the instrument will automatically check its zero positions (the monochromator motor will be audible) and perform an electronics self-test procedure. If the self test procedure fails, the green Status LED will flash indicating an electronics error. In the event of a self-test failure, restart the Electronics Unit to remedy the fault. If the fault persists, please contact the Technical Support Team at Applied Photophysics.

The two red LEDs labelled Tx and Rx indicate that the Electronics Unit is transmitting and receiving information respectively.

The Electronics Unit is switched off by moving the rocker switch to the off (O) position.

2.4.7.3 Maintenance

The Electronics Unit contains no user serviceable components and should not require any maintenance. In case of a damaged fuse, the 1A mains fuse holder is located above the mains inlet socket.

2.4.7.4 Electrical Characteristics

The electrical characteristics of the system are as follows:-

Supply voltage	*85-264V*
Supply frequency	*47-63Hz*
Power rating	*80VA*
Fuse	*1A*

2.4.8 Workstation

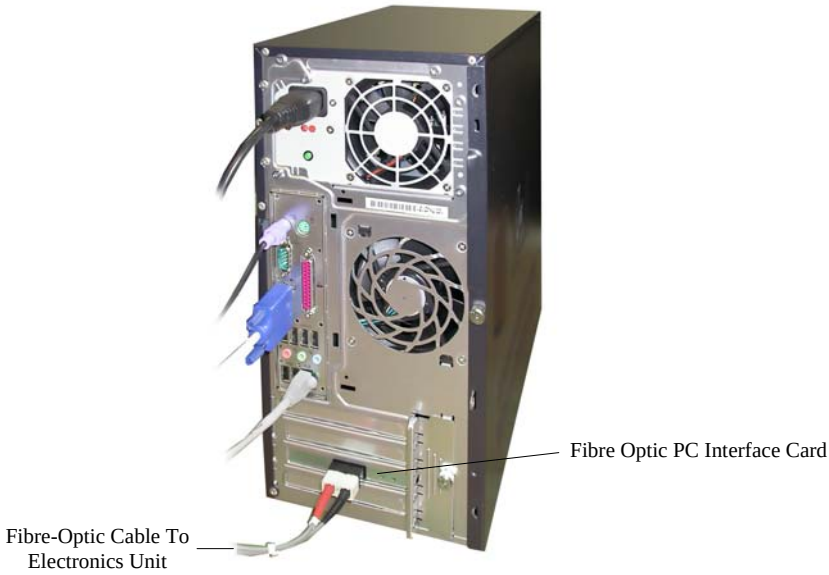
2.4.8.1 Description

The SX20 features a Hewlett Packard PC fitted with a fibre-optic interface card that is linked to the Electronics Unit of the SX20 and provides computer control of the stopped-flow data acquisition via the SX Control Software.

The PC is connected to a flat-screen monitor, ink-jet printer and network socket. A programmable water circulator may also be connected to provide temperature control of the stopped-flow unit.

2.4.8.2 Basic Set-Up and Electrical Connections

The set-up of the computer is fully described in the Hewlett Packard PC user manual supplied with the computer. Please refer to this manual for detailed instructions for setting up the computer. The interface card is connected to the Electronics Unit via a fibre-optic cable.



2.4.8.3 Operation

Please refer to the Hewlett Packard user manual supplied with the computer for full details on operation of the computer. The operation of the SX20 via the workstation is described fully in the software chapters of the User Manual.

2.4.9 Photo Diode Array Detector (Option PDA.1)

2.4.9.1 Description

The SX20 may be equipped with an optional photodiode array detector for collection of time-resolved absorbance spectra from a single stopped-flow drive.

There are two versions of the 256 element photodiode array accessory: The visible region PDA has an operational spectral range of 300-1100nm with a wavelength resolution of 3.3nm. The UV region PDA has an operational spectral range of 190-735nm with a wavelength resolution of 2.2nm. Each PDA is capable of an integration speed of 1.4ms per scan.

2.4.9.2 Basic Set-Up and Electrical Connections

The cream coloured photodiode array unit is normally positioned on the rear of the sample handling unit. The PDA is connected to the cell housing via a fibre-optic light guide. The light guide is attached to the cell housing and the PDA via two finger screws. The light guide has a rectangular bundle of fibres of dimensions 7mm x 1mm at the SHU end and a circular bundle of fibres of 3.5mm diameter at the PDA end.

The PDA unit is connected to the PDA module in the Electronics Unit via a black cable fitted with 26-pin D-connectors. The yellow diode labelled 256 will be illuminated when a PDA is attached.

2.5 SX20 Experimental Operation

This section provides detailed information relating to experimental operation of the SX20 stopped-flow reaction analyser hardware.

2.5.1 Single Mixing Stopped-Flow Operation

The following procedure describes the use of the syringes and the various valves during sample loading, sample flow circuit flushing and stopped-flow mixing. This is the basic experimental template for stopped-flow operation. Variations of this template will be described in a later section of this chapter.

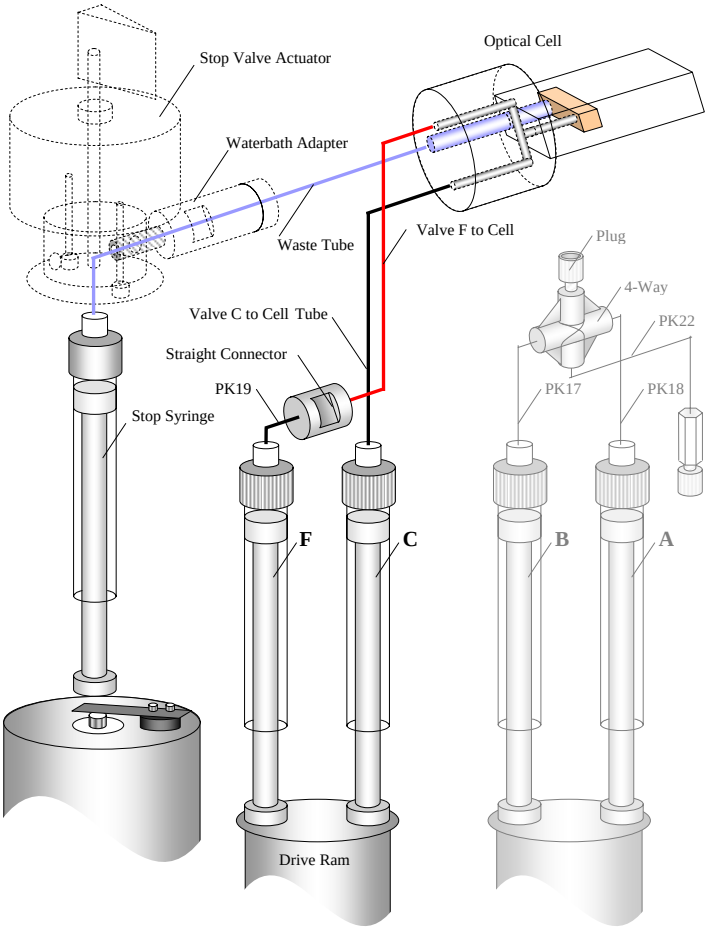
STEP 1 Sample Handling Unit Preparation (Sequential Mixing Systems Only)

For systems equipped with the sequential mixing option (SQ.1), the sample handling unit should be set-up as shown in the diagram below with the single mix drive ram connected to the left hand ram platform. The single mixing experiment always utilises this drive ram and the reagents are always loaded in the F (left) and C (right) drive syringes. The black plastic dust cover should be fitted to the right hand (Pre-Mix) ram platform.

STEP 2 Flow Circuit Configuration (Sequential Mixing Systems Only)

For systems equipped with the sequential mixing option (SQ.1), the flow circuit should be set-up as shown in the diagram below with the flow tubing connecting directly between drive valve F and the optical cell.

Systems not equipped with the sequential mixing option are only configured for single mixing experiments. No additional preparation is required.



STEP 3 Adjusting the Drive Volume

Screw the knurled volume adjuster on the Auto-Stop fully clockwise (i.e. front edge moves from left to right), then screw it three and a half turns anti-clockwise (counter-clockwise). This equates to a total flow volume of approximately 140 µL.

STEP 4 Flushing the Flow Circuit

Fit two reservoir syringes filled with distilled water to the C and F luer fittings. It is recommended that 5 or 10mL plastic disposable syringes be used.

Turn the C and F drive valves to the *Load* (side) position. Ensure that the drive ram is pushed fully down. Fill the C and F drive syringes by pushing down the plungers of the reservoir syringes. Expel any air bubbles by flushing backwards and forwards between the drive syringes and the reservoir syringes several times.

Turn the F drive valve to the *Drive* (forward) position and manually push the water from the drive syringe, through the flow circuit and into the stop syringe.

Empty the stop syringe by clicking on *Empty* in the Pro-Data control software.

Repeat the above two steps until the drive syringe is empty.

Turn the F drive valve to the *Load* (side) position and the C drive valve to the *Drive* (forward) position and manually push the water from the drive syringe, through the flow circuit and into the stop syringe.

Empty the stop syringe by clicking on *Empty* in the Pro-Data control software.

Repeat the above two steps until the drive syringe is empty.

If there are bubbles in the stop syringe that cannot be expelled by manual pushing, the syringe must be removed and the bubbles removed. To collect quality data it is essential that air bubbles are not present in the flow circuit.

STEP 5 Loading the Reagents

Ensure that all the control valves are set to the *Load* (side) position. Replace the C and F reservoir syringes with ones containing the samples. Where applicable, the solution of greatest density **must** be loaded in the F drive syringe. If both samples are of the same density then the most precious sample, should be loaded in the C syringe as it has the smaller priming volume.

Fill the drive syringes by pushing down the pistons of the reservoir syringes (this ensures that there is no cavitation inside the drive syringes and, therefore, no bubbles).

Prime the sample handling unit by manually pushing 200µL from each drive syringe.

Completely fill both of the drive syringes and ensure that there is no gap between the drive ram and the drive syringe plungers.

Set both drive valves to the *Drive* (forward) position.

STEP 6 Data Acquisition

The experimental procedures for the various modes of data acquisition are described in detail in the SX20 Operation chapter of this User Manual.

Twenty-five drives can be made in quick succession before the drive syringes will need to be refilled.

2.5.2 Sequential Mixing Stopped-Flow Operation

The sequential mixing stopped-flow experiment allows investigation of the reactivity of reaction intermediate species.

The following procedure describes the use of the syringes and the various valves during sample loading, sample flow circuit flushing and sequential stopped-flow mixing.

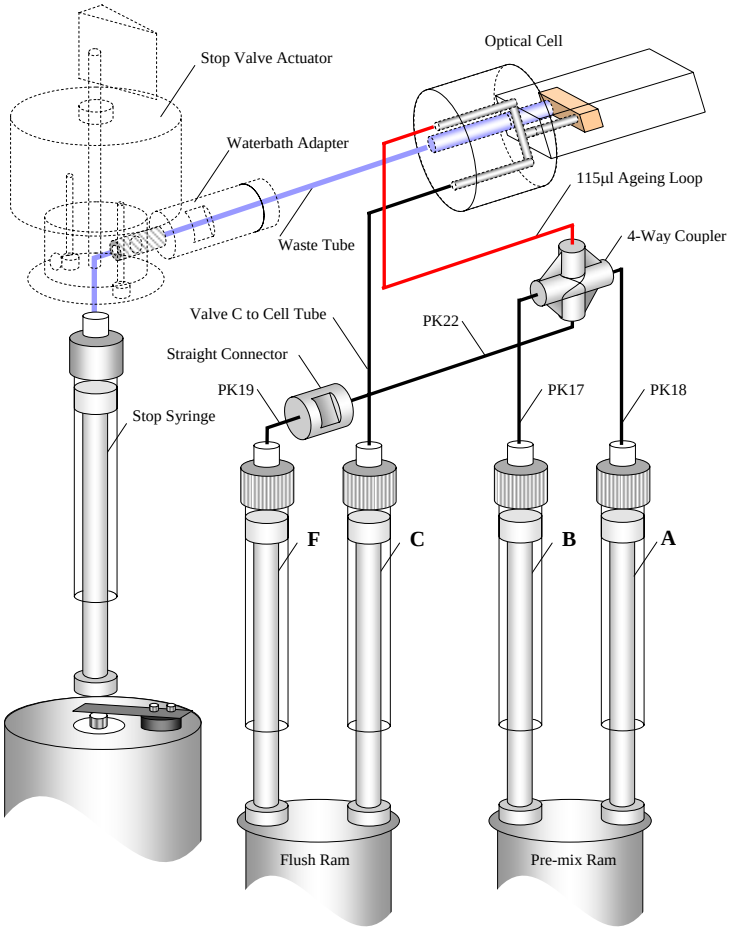
The sequential mixing experiment employs a double pneumatic drive. The first (Pre-Mix) drive mixes the contents of the two right hand drive syringes (A and B) in an aging loop. After a user defined aging period (from 0.010s to 1000s) the second (Flush) drive mixes the contents of the aging loop with a third reagent C.

STEP 1 Sample Handling Unit Preparation

The sample handling unit should be set-up as shown in the diagram below with the flush drive ram connected to the left hand ram platform and the pre-mix drive ram connected to the right hand ram platform.

STEP 2 Flow Circuit Configuration

The flow circuit should be set-up according to the following instructions and referring to the diagram below:-



Drain the thermostat solution from the water bath by removing the bleed screw from the top of the cell block.

Remove the front cover of the water bath.

Remove the aging loop from the straight through connector that connects to the flush line. Remove the plugs from the pre-mixer and the end of the extension tube.

Connect the extension tube to the straight through connector on the end of the flush line and the aging loop to the pre-mixer. Pressure test the flow line to make sure that it is not leaking. Replace the front of the water bath and the bleed screw.

STEP 3 Adjusting the Pre-Mix Drive Volume

Screw the fine adjuster on the pre-mix drive ram fully upwards so that the ram will have no upward travel (this is the zero drive volume position). Screw the fine adjuster five turns to increase the drive volume. This equates to a pre-mix drive volume of approximately 220µL. It is essential that the pre-mix drive volume should be 220µL±10µL.

Use of the Profiles function in the Pro-Data control software allows accurate measurement of the drive volume and will be described in STEP 6.

STEP 4 Adjusting the Total Drive Volume

Screw the volume adjuster on the Auto-Stop fully clockwise (i.e. front edge moves from left to right, until the pneumatic connection starts to rotate), then screw it eight and a half turns anti-clockwise (counter-clockwise). This equates to a total drive volume of approximately 400µL and will result in a drive 2 volume of 180µL (±10µL) assuming a pre-mix (drive 1) volume of 220µL (±10µL).

Use of the Profiles function in the Pro-Data control software allows accurate measurement of the drive volume and will be described in STEP 6.

STEP 5 Flushing the Flow Circuit

Fit four reservoir syringes filled with distilled water or another suitable solvent to the A, B, C and F luer fittings. It is recommended that 5mL or 10mL plastic disposable syringes are used.

Turn the A, B, C and F drive valves to the *Load* position. Ensure that the drive rams are pushed fully down. Fill the drive syringes by pushing down the plungers of the reservoir syringes. Expel any air bubbles by flushing backwards and forwards between the drive syringes and the reservoir syringes several times.

Turn the F drive valve to the *Drive* position and manually push the water from the drive syringe, through the flow circuit and into the stop syringe.

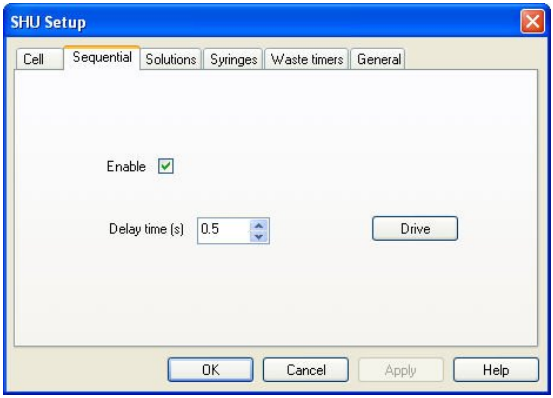
Empty the stop syringe by clicking on *Empty* in the Pro-Data control software.

Repeat the above two steps until the drive syringe is empty.

Flush from the other three drive syringes in the same manner as described above.

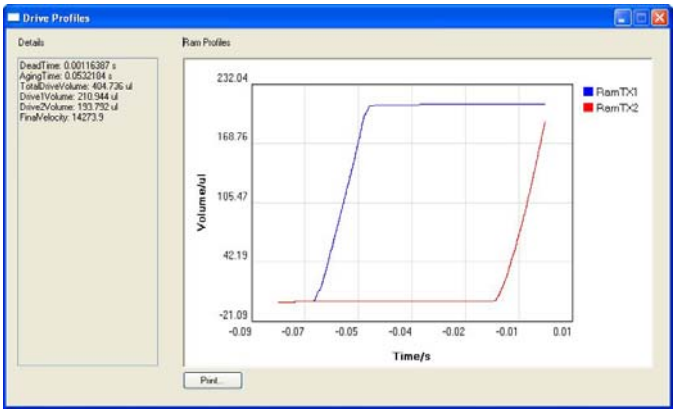
Fill all four drive syringes with water and turn all of the drive valves to the *Drive* position.

Enable the sequential mixing mode in the Pro-Data control software. With a delay time of 1s, carry out a stopped-flow drive by clicking the *Drive* button in the window shown below. The drive profiles will be displayed automatically after the drive and should be used to check the accuracy of the drive 1 and 2 volumes.



STEP 6 Checking the Drive Volume Using the Profiles Function

Carry out several drives and check that the drive 1 volume is 220µL (±10µL) and the drive 2 volume is 180µL (±10µL) from the Drive Profiles data shown in the window below. The drive volumes and measured delay time should be consistent from run to run.



STEP 7 Loading the Reagents

Ensure the all four drive valves are set to the *Load* position. Replace the reservoir syringes with ones containing the samples. The F (flush) reservoir syringe should be filled with a neutral buffer solution. Fill the drive syringes by pushing down the pistons of the reservoir syringes (this ensures that there is no cavitation inside the drive syringes and, therefore, no bubbles). Ensure that there are no air bubbles in the drive syringes.

Set the four drive valves to the *Drive* position.

STEP 8 Data Acquisition

The experimental procedures for the various modes of data acquisition are described in detail in the Operation Manual chapter of this user manual.

The first trace acquired with new reagents will prime the flow lines and should be discarded. The drive syringes must be refilled after each acquisition.

2.5.3 Optical Filters

The SX20 is designed to operate with a variety of optical filters according to experimental requirements.

2.5.3.1 Absorbance Measurements in the near IR (>650nm)

It is essential that absorbance measurements collected at wavelengths greater than 650nm use an appropriate filter to eliminate second order stray light from detection. Second order stray light is a phenomenon common to all monochromators fitted with diffraction gratings. In addition to monochromatic light of the required wavelength, a small proportion of light with half this wavelength is also generated.

All SX20 systems are supplied with a 645nm cut-off filter that should be positioned between the optical cell and the absorbance detector when measuring absorbance in the near IR region.

2.5.3.2 Fluorescence Measurements

Optical filters are used in fluorescence measurements to eliminate the scattered excitation light from detection by the fluorescence PMT. The fluorescence signal is measured at a 90^{o} angle to the excitation light and the filter is mounted between the optical cell and detector.

The filter (25mm diameter) is mounted in the circular filter holder and retained in position using the split ring. The filter holder shown below is designed for use with cut-off and band-pass filters.

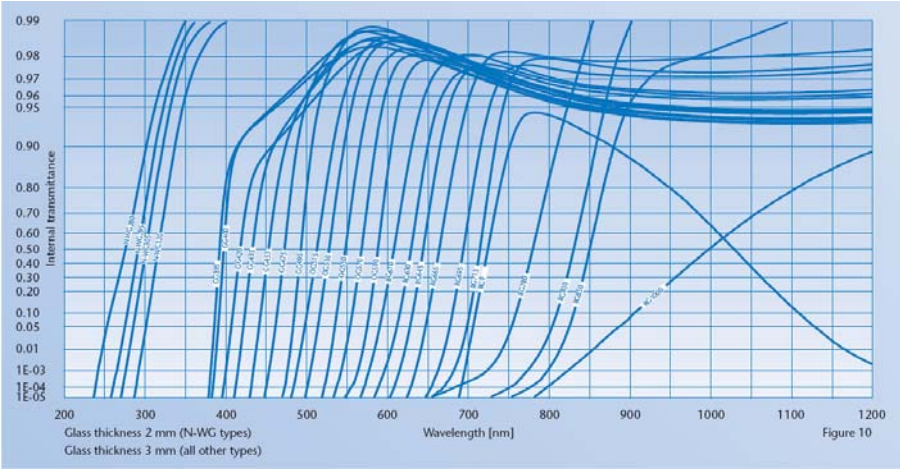
Filter Holder

Cut-Off Filters

Cut-off filters eliminate light of shorter wavelength than its cut-off value. Applied Photophysics stock the following range of Schott manufactured filters suitable for use with the SX20.

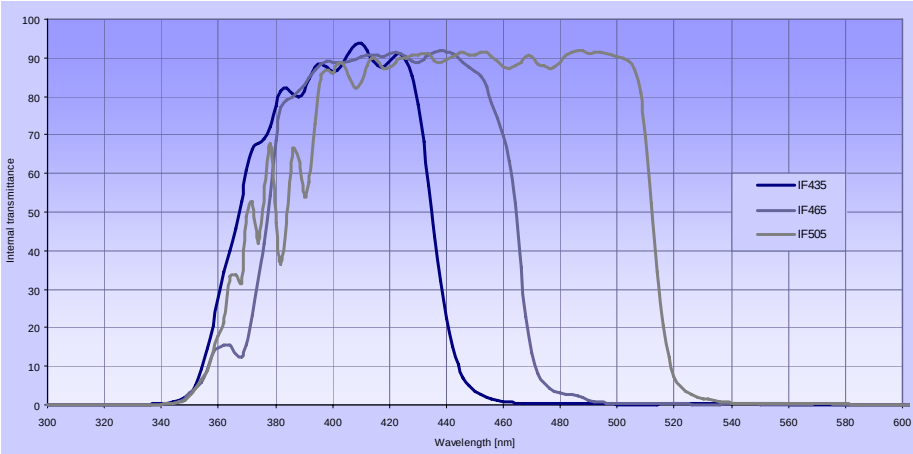
295	375	475	570
305	395	495	590
320	400	515	610
335	420	530	630
360	455	550	645

The transmittance profiles for these filters are displayed below:



Band-Pass Filters

Band-pass filters transmit a band of light of a certain wavelength. Band-pass filters are most suited to applications where more than one fluorophor is present in the reaction mixture. Applied Photophysics offer a 435, 465 and 505nm filters for use with the SX20. The transmittance profiles for these filters are displayed in the diagram below:



2.5.4 Asymmetric (Variable Ratio) Mixing

The SX20 is fitted with 2.5mL Kloehn manufactured drive syringes as standard. This provides a 1:1 stopped-flow mixing ratio. Other mixing ratios may be achieved by fitting drive syringes of different volumes to the sample handling unit.

For single mixing stopped-flow measurements the following ratios may be achieved using one standard 2.5mL Kloehn syringe and one of the Hamilton manufactured Salt Line syringes.

Mixing Ratio	Syringe Configuration
1:1	2.5mL + 2.5mL
2.5:1	2.5mL + 1mL
5:1	2.5mL + 500µL
10:1	2.5mL + 250µL
25:1	2.5mL + 100µL

2.5.4.1 Setting the Drive Pressure for Asymmetric Mixing Experiments

Caution! To prevent damage to the flow circuit from excessive pressures produced when using drive syringes smaller than the standard 2.5mL Kloehn syringe, the pneumatic drive pressure must be reduced via the pressure regulator fitted to the rear of the sample handling unit.

The drive pressure is set at 4bar for standard 1:1 mixing experiments. For 1:5, 1:10 and 1:25 mixing ratios the drive pressure **must** be reduced to 2 bar using the regulator connected to the gas inlet on the rear of the sample handling unit.

2.5.4.2 Setting the Drive Volume for Asymmetric Mixing Experiments

The total drive volume should be increased as the ratio increases in order to obtain good reproducible results. The table below lists some recommended drive volumes that may be used as a guide during experimental set-up.

Mixing Ratio	Drive Volume
1:1	140µL
2.5:1	160µL
5:1	200µL
10:1	250µL
25:1	300µL

Preparing the SHU for Asymmetric Mixing

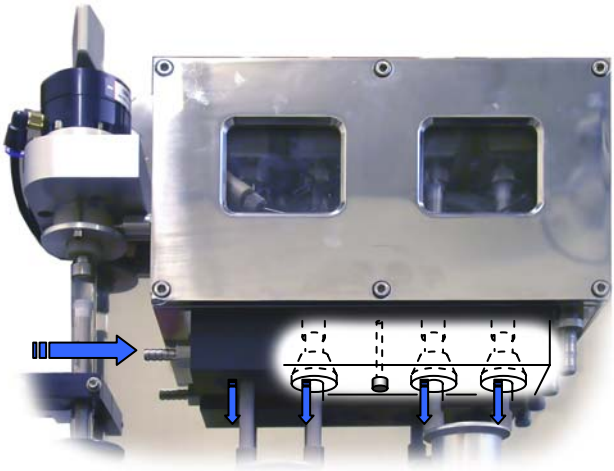
1) Check that the drive pressure has been set to 2bar.
2) Drain the water bath housing and remove the front plate.
3) Unscrew the F drive syringe from the drive valve retaining plate by gripping the metal tip. Withdraw the syringe down through the rubber water bath seal.
4) Repeat the reverse of the removal procedure to fit the Hamilton syringe. It will be necessary to fit a rubber sleeve to the glass barrel of smaller diameter syringes to prevent water bath leaks. Tighten the syringe into the drive valve retaining plate carefully to provide a leak-free fit.
5) Test the flow circuit for leaks using the procedure described previously and tighten the drive syringe further if necessary.
6) Replace the front plate of the water bath housing.
7) Adjust the drive volume on the Auto-Stop according to the values listed in the table above.

2.5.5 Anaerobic Operation (using option AN.1)

The AN.1 anaerobic accessory equips the SX20 with a high performance bench top anaerobic capability. In such an environment, operating under anaerobic conditions requires stringent care with respect to eliminating oxygen dissolved in the thermostat medium and adsorbed into the wall material of the sample flow circuit. The accessory comprises a purging manifold and a set of 3-way valves.

2.5.5.1 Purging the Drive Syringes

The most significant source of oxygen ingress is between the plunger and barrel of the drive syringe. Although the syringes are gas tight, the tips of the plungers are made of Teflon which does not act as a barrier to oxygen. In order to stop oxygen from passing between the barrel and plunger, the end of the syringe barrel needs to be maintained in an inert environment. This is achieved using the anaerobic purging manifold shown attached to the sample handling unit in the picture below.



To fit the accessory, push it over the drive syringe plungers and screw the accessory to the underside of the sample handling unit water bath so that the ends of the drive syringe plungers are enclosed. Connect an inert gas supply (nitrogen or argon) to the male fitting on the side of the manifold and gently purge so that the ends of the drive syringes are in an inert environment. The previous diagram shows a cut away view of the assembled accessory.

2.5.5.2 Flow Circuit Preparation

Another significant source of oxygen contamination is through the flow circuit. The SX20 sample handling unit is fitted with PEEK tubing and PEEK drive valves as standard. This minimises the danger of oxygen contamination from the tubing and the valves.

The thermostatic liquid in the circulator should be purged of oxygen by gently bubbling nitrogen through it at 25°C ± 1°C. 1g of sodium dithionite should be added to the thermostat liquid. The circulator must be left running for the duration of anaerobic operation. A solution of 600ng/ml Glucose Oxidase and 10mM glucose in 100ml of sodium acetate, pH5, should be flushed through the internal flow lines of the sample-handling unit. After 1 hour deoxygenated buffer should be used to flush out the Glucose Oxidase solution. The sample handling unit is now ready for anaerobic use.

2.5.5.3 Sample Introduction Care

Another source of oxygen contamination is at the point where the samples are introduced into the drive syringes. For basic anaerobic work the use of gas tight luer tip reservoir syringes is recommended. Prepare the anaerobic samples as usual, draw them into gas tight syringes, bring them quickly to the sample handling unit, as you are about to fit them to the luer connections expel a small amount of the sample to prevent an air bubble from being trapped between the reservoir syringe and the fitting.

Advanced anaerobic work requires the use of three-way valves to introduce the samples into the system. These are supplied as part of the AN.1 accessory and may be used according to the following procedure:

2.5.5.4 Procedure for Bench Top Anaerobic Stopped-Flow Studies

1) Flush the drive syringes and flow circuit of the SHU with anaerobic buffer. After flushing, a little anaerobic buffer should be left in the drive syringes.
2) Prepare all reagents in a glove box and load these into gas-tight (luer lock) syringes with a 3-way stop valve on the end of the syringe, ensure this valve is closed.
3) The syringe and 3-way valve should be transferred to the stopped flow instrument.
4) Connect an empty disposable syringe on to the unused port on the 3-way valve. With the 3-way valve in the same position (i.e. closed), turn the drive valve to the *Load* position and push up on the drive syringe to expel the anaerobic buffer from the drive syringe into the empty disposable syringe on the side of the 3-way valve. This effectively scrubs the drive valve, luer fitting and part of the 3-way valve making these components anaerobic.
5) Open the 3-way valve by turning it 90 degrees and introduce a small amount of the anaerobic sample into the drive syringe.
6) Turn the 3-way valve back to the closed position and then push this small volume of anaerobic sample out through the 3-way valve to the disposable syringe (containing anaerobic buffer). This fills the valve, syringe and luer fitting with sample.
7) Open the 3-way valve and load the sample into the drive syringes. The system is now ready for the stopped-flow experiment.